PROGRESS REPORT -- 2000

CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE

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This report includes results of research in progress. It is not intended for publication, and should not be referred to in literature citations.

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The overall goal of the research program of the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) is to develop integrated management technologies and strategies for insects and other arthropods of agricultural, medical and veterinary importance. This report provides abstracts of research in progress and is not intended for citation in any publication. Reprints of published articles may be obtained by contacting the individual authors.

RECOGNITION and HONORS

Two CMAVE employees received U.S. Department of Agriculture Awards from the Secretary of Agriculture, Mr. Dan Glickman, at a ceremony in Washington, D. C., June 5, 2000. Dr. Richard Brenner, Research Leader of the Imported Fire Ant and Household Insects Research Unit, received an award for development and leadership of a research program to enhance the environment and human health through reduction of pesticide use, and precision targeting of pest control measures. Ms. Bonnie Ebel, Secretary to the Center Director, received an award for outstanding service and impact on organizational efficiency and productivity.

Dr. James Tumlinson was selected by the Entomological Society of America for the 2000 Recognition Award in Physiology, Biochemistry and Toxicology for his research on the chemical and biochemical basis of olfaction and behavior in insects.

Dr. Daryl Pring was awarded a 2001 ARS-Headquarters Postdoctoral Research Associate Fellow for his research program in the Crop Genetics and Environmental Research Unit.

STAFF and ORGANIZATIONAL CHANGES

Three scientists were added during the past year to the permanent staff of CMAVE. Dr. Stephen Hight joined the Behavior and Biocontrol Research Unit and is now conducting research at the Center for Biological Control at Florida A&M University in Tallahassee, Florida as part of our cooperative research program on biological control of crop pests and weeds. In addition, Dr. Eric Schmelz joined the Chemistry Research Unit, and Dr. Uli Bernier joined the Mosquito and Fly research Unit. Also, Dr. Daniel Wojcik retired from CMAVE after a productive career in research on the imported fire ant.

Effective October 1, 2000 the mission of the CMAVE was broadened to include the Crop Genetics and Environmental Research Unit, Dr. Prem Chourey, Research Leader. This group is housed in University of Florida research facilities in close proximity to CMAVE. The CGERU research program will be reported in the 2001 annual report. Also, effective October 1, 2000 Herbert Oberlander became full-time Center Director, creating a vacancy for Research Leader of the Postharvest and Bioregulation Research Unit.

Herbert Oberlander
Center Director

Center for Medical, Agricultural, and Veterinary Entomology

Agricultural Research Service
United States Department of Agriculture

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H. Oberlander, Center Director
B. Ebel, Secretary

MISSION

The Center conducts research on insects of agricultural, medical and veterinary importance with the goal of achieving control of pest species through the development of environmentally acceptable approaches. Emphasis is placed on developing components and systems for integrated pest management, based upon an understanding of the behavior, physiology and ecology of pest species. Sensitive detection devices that employ semio-chemicals and electronic technology will provide the means for early intervention. Investigations will lead to biological control based on parasites, predators and microbes, and thus provide alternative, biorational tools for managing populations of pest species. Special attention is focused on insect pests of field and horticultural crops, stored products and on arthropod pests of medical and veterinary importance. Protection of humans from arthropods of medical importance is a continuing priority. The scope of the Center's research is national and international and impacts agricultural production, postharvest storage and transport of agricultural commodities, and protection from household and disease carrying arthropods. Research is conducted to meet the needs of state and federal regulatory agencies, the Department of Defense, industry, universities, growers, commodity groups and the public at large.

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This Unit conducts research on insect behaviors that regulate reproduction, feeding, foraging and migration. Principles of behavior are emphasized, especially reproductive behavior of pest and beneficial insects. Results of this research are applied directly to control programs and technology, such as genetic eradication programs against Mediterranean fruit flies in Central America and Caribbean fruit flies in Florida, and integrated pest management of lepidopterous pests of field and vegetable crops. The CMAVE Scientists at Florida A&M University in Tallahassee, Florida conduct research on biological control of weeds and insect pests of vegetable crops.

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This Unit investigates the chemical, biochemical and physiological factors that regulate insect behavior and the interaction of insects with plants and other organisms in the environment. The research program focuses on the following major areas: identification, synthesis, and behavioral evaluation of pheromones that regulate mating and other behaviors of important insect pests; identification, synthesis and behavioral evaluation of kairomones and other semiochemicals that influence the foraging behavior of beneficial entomophagous insects; identification, synthesis and behavioral evaluation of plant-produced chemicals that influence the behavior of insects; and elucidation of the biochemical mechanisms that regulate insect pheromone production, release and perception.

Crop Genetics and Environmental Research

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This Unit conducts research on crop plants to identify and characterize genes of agronomic importance, to assess the impact of global climate change on future crop productivity, to improve biological nitrogen fixation and to identify alternatives to methyl bromide for protection of crops from soil borne pests.

Imported Fire Ant and Household Insects

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This Unit develops reduced-risk integrated management strategies for cockroaches and their attendant allergens, pest ants, fire ants and, through cooperative agreements, termites. Areas of research include insecticide detoxification mechanisms; spatially-based risk assessment and insect behavioral ecology pertaining to the development of baits, repellents, and biological control agents; population dynamics; sociobiology of insects; bioecology and biodiversity; and pheromone chemistry and chemical ecology.

Mosquito and Fly

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Research in this Unit results in new arthropod repellents and personal protection technology for the US military and new detection, population monitoring, and control technology for biting and filth breeding insects, ticks, and mites.

Postharvest and Bioregulation

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This Unit conducts research on the detection, population estimation and control of stored product insects. New detection tools are developed based on acoustical and electronic methods, as well as chemical ecology. Research approaches to population management include the application of insect behavior, molecular biology, and biochemistry to the control of growth and development of these insects.

EXAMPLES OF RECENT RESEARCH

Parasites Smell Success

Parasites and predatory arthropods often prevent plants from being severely damaged by killing herbivores as they feed on the plants. A breakthrough in understanding how these biological control agents locate their insect hosts was achieved with the isolation and identification of a volatile chemical, "volicitin", obtained from the oral secretions of beet armyworm caterpillars. When applied to damaged leaves of corn seedlings, volicitin induced the seedlings to emit volatile compounds that attract parasitic wasps which are natural enemies of the caterpillars.

Mosquitoes Find New Traps Alluring

A new trap lures mosquitoes with a bland of chemicals that mimic the national attractants. It has been named "Dragon Fly" for the insect that is known as a mosquito hunter, and is currently being commercialized.

Genes on the Move

The ability to insert genes into *Drosophila* suggested opportunities for a new approach to insect control. Progress in this area, however, has been held up by a lack of genetic transposons that would allow scientists to insert genes of choice in other insect species of economic importance. Recent experiments with both fruit flies and moths have been very successful. For example, a new transposon, *piggybac*, can transform the Mediterranean fruit fly, as well as other economically important insects.

Taking a Bite out of Fire Ants

When Imported fire ants were introduced into the United States, almost all of their natural enemies remained behind in South America. Efforts to introduce biological control of fire ants have led to the first release in the United States of a South American phorid fly. Fly eggs hatch into larvae in the fire ants, and have the peculiar effect of decapitating the host. Then, the flies complete their development in the severed head capsule until they emerge as adult flies. Phorid flies have been successfully released, and show promise for contributing to reduced fire ant populations.

Houseflies Succumb to Worms

Adult houseflies that develop from larvae infected with parasitic nematodes lived only half as long as uninfected flies. This nematode species, originally collected from Brazil, has potential as a biological control agent for houseflies because it appears to be host specific and can be raised easily in large quantities. Moreover, because there are few natural enemies that attack flies in the larval stage this nematode may be compatible with other biological control agents.

Eavesdropping on Insects

A highly sensitive electronic detection device was developed for stored grain insects. A grain probe trap was modified by incorporating a sensor head with infrared electronics so that insects that enter the trap can be electronically sensed and counted. This detection method may be applicable to a wide variety of insects, and is currently being commericalized.

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AND

BIOCONTROL

CRIS - 6615-22000-014-00D--Biological Control Through Artificial Rearing of Natural Enemies and Manipulation of Host Plant Resistance

CRIS - 6615-22000-015-00D–Development of Biologically Based Mechanisms of Control of Insect Pests of Fruit, Vegetable, Field Crops

CRIS - 6615-22000-016-00D–Biologically-Based Technologies for Management Crop Insect Pests in Local and Area Wide Programs

CRIS 6615-22000-014-00D

BIOLOGICALLY BASED PEST MANAGEMENT THROUGH ARTIFICIAL REARING OF NATURAL ENEMIES AND MANIPULATION OF HOST PLANT RESISTANCE

S.M. Ferkovich, and J.P. Shapiro

Objective: Commercial producers of beneficials would be more inclined to rear *Orius insidiosus* (Hemiptera: Anthocoridae) on a larger scale if an economical artificial diet and oviposition substrate were available to facilitate rearing the insect. Our objectives were to a) supplement two existing artificial diets to improve fecundity, b) determine the digestibility of commercial proteins added to the diet and their effects on fecundity, and c) develop an artificial substrate for egg deposition.

Methods: Two published artificial diets, one with a chicken egg yolk base/hydrolyzed soy protein and another with a chicken yolk/beef liver base were each supplemented with whole eggs of *Plodia interpunctella* and protein and lipid extracts from homogenized *Plodia* eggs. *Plodia* eggs were homogenized and extracted with chloroform:methanol (2:1 v/v) and after drying down the chloroform and methanol phases separately, the residues from each solvent phase were evaluated in the artificial diet. Cellular fatty acids were identified in the lipid extract by gas chromatography and the appropriate ratios of predominant fatty acids were added to the diets. Supernatants from homogenized eggs were also run on a gel exclusion column and concentrated by freeze drying then added to the diets. Commercial proteins were mixed in Tris-HCL buffer, centrifuged then incubated with supernatants from homogenized midguts from females of *Orius insidiosus*; digestive products were examined on polyacrlamide gels. Artificial oviposition substrates composed of gelled droplets were treated with three vegetable and fruit coatings, Certiseal A-100, Natureseal CA-1 and Pharmaceutical Glaze (# 3.5-10) to prevent dehydration of the fdroplets and were presented to *Orius* females for oviposition.

Results: The data is in the midst of statistical analysis and it is too early to draw specific conclusions about all the data. *Orius* can be reared on the two artificial diets; however, fecundity is significantly lower than that of the females fed on whole *Plodia* eggs. Homogenized *Plodia* eggs added to the liver/yolk-based diet increased egg production 7%, but production is still 50% of the control (whole *Plodia* eggs). The addition of protein and lipid extracts from *Plodia* eggs to the chicken yolk/soy protein-based diet enhanced egg production 25% and 37%, respectively over diet alone but production was still 42% and 52%, respectively of that observed on whole *Plodia* eggs. Addition of the predominant fatty acids identified in *Plodia* eggs to the yolk/soy protein-based diet boosted egg production 9% over diet alone and 32% of the control (*Plodia* eggs). Soluble proteins from solutions of egg albumin, bovine fetuine, beef liver, hemoglobin, BSA, and brewers yeast were all digested by whole extracts of *Orius* midguts to varying degrees but the results were inconclusive. Substituting chicken liver for beef liver enhanced egg production from 20% to 60% of the control (*Plodia* eggs). *Orius* females did not lay eggs in the gel droplets coated with any of the materials although they did oviposit in droplets coated with parafilm.

CRIS 6615-22000-014-00D

IMMUNOSORBENT ASSAYS (ELISAs) TO ASSESS REPRODUCTIVE QUALITY AND IMPROVE DIET DEVELOPMENT FOR INSECT PREDATORS

J.P. Shapiro and S.M. Ferkovich

Objective: To develop yolk polypeptide-based ELISAs as tools to rapidly assess the reproductive state of female insect predators. ELISAs will be employed 1) as a tool to monitor the quality of insects produced in insectaries; 2) to quickly assess the impact of dietary supplements on reproduction; and 3) to assess reproductive fitness in augmented field populations.

Methods: Hybridomas and monoclonal antibodies were produced from the cells of mice that had been immunized with homogenized eggs of the heteropterans *Podisus maculiventris* (Pentatomidae) and *Orius insidiosus* (Anthocoridae). Hybridoma cell lines were selected by screening mass cell cultures for cross-reactivity against eggs and female hemolymph (*P. maculiventris*), or female homogenates (*O. insidiosus*) and against cross-reactivity to males. Indirect ELISAs were developed and validated for reactivity against eggs, hemolymph (*P. maculiventris* only), and whole-insect homogenates.

Results: For *P. maculiventris*, these methods resulted in production of mAbs that selectively reacted only against a 171,000 M_r polypeptide in fed females, their eggs, and hemolymph, but not against males (Shapiro et al., 2000). For *O. insidiosus*, mAbs also reacted specifically against female homogenates and eggs. However, the mAb initially selected for use in *O. insidiosus* ELISAs, 5C2-1F3, reacted strongly against a 41,000 M_r polypeptide in eggs and females, and an additional band at about 56,000 M_r in females (Fig. 1).

The ELISAs developed with these mAbs consistently detected vitellin in egg extract equivalent to that in 10-1000 ng of total soluble egg protein, in either *P. maculiventris* or *O. insidiosus* (Fig. 2). A time course of titers in *P. maculiventris* hemolymph and whole bodies demonstrated the concept that an ELISA can predict clear differences between females fed on optimal and suboptimal diets (Fig. 3).

Figure 1. Egg and hemolymph proteins from female and male *O. insidiosus* on a Western blot reacted with mAb 5C2-1F3 and on a duplicate SDS polyacrylamide gel (SDS-PAGE).

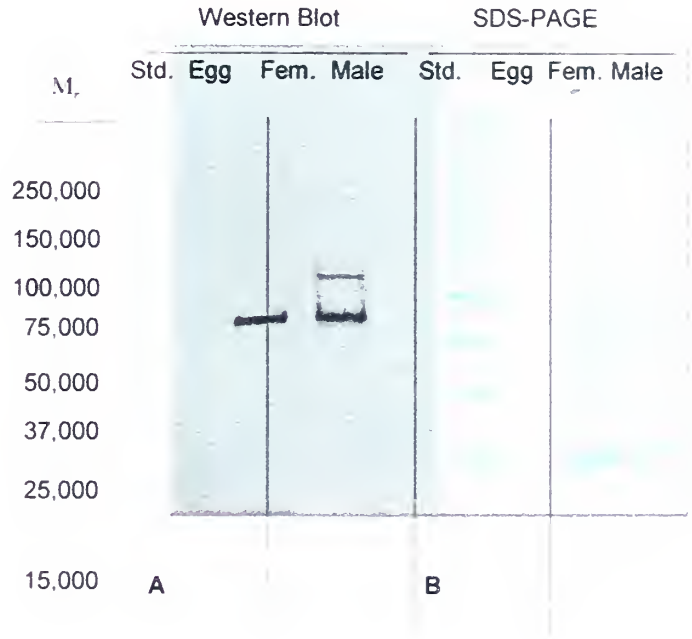


Figure 2. Standard curves from ELISAs for (A) *P. maculiventris* and (B) *O. insidiosus*.

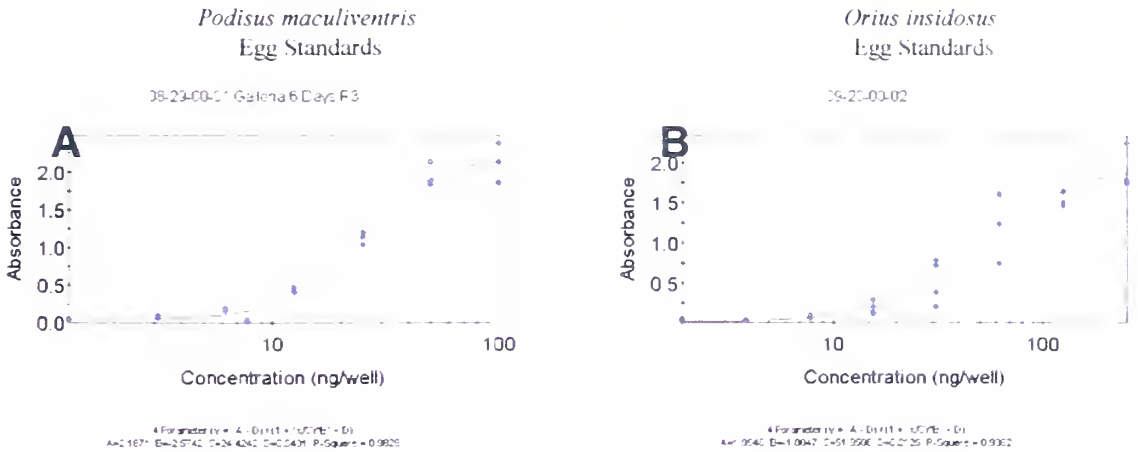
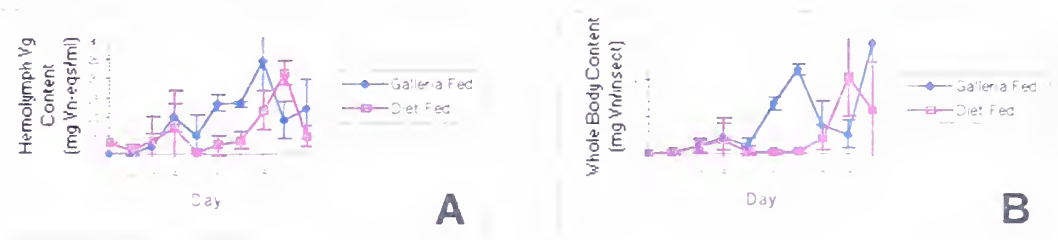


Figure 3. Time course of egg development in *P. maculiventris*. Yolk protein content of (A) hemolymph and (B) whole extracts of females fed on *Galleria* larvae or an artificial diet.



CRIS 6615-22000-015-00D

WITHIN PLANT DISTRIBUTION OF *FRANKLINIELLA* THRIPS IN TOMATOES

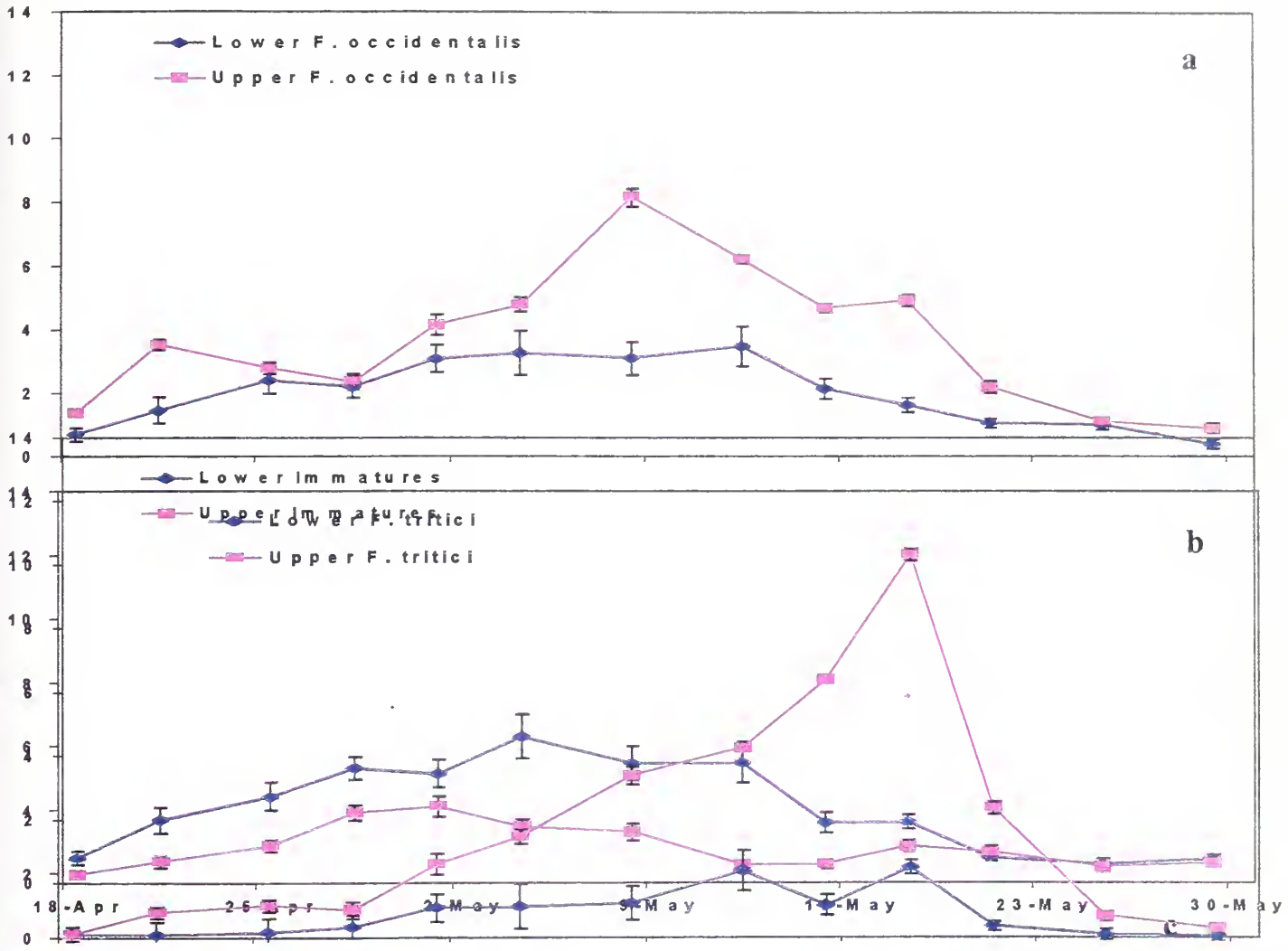
S. Reitz

Objectives: Several species of thrips comprise one of the primary pest complexes of tomatoes and other vegetables in the southeast. The objectives of this research are to determine the seasonal dynamics and within plant distribution of these thrips in tomatoes grown under different fertilizer regimes. This research is designed to improve the understanding of the species specific ecology of and population dynamics of *Frankliniella* thrips and develop more efficient sampling protocols for thrips in vegetable crops.

Methods: Tomato plants were transplanted on March 18 at the Florida A&M University Research and Education Center, in Quincy. The field was laid out with eight blocks and three plots per block. Plants were grown on black plastic mulch and were drip irrigated. Three nitrogen fertilizer treatments (sub optimal 90kg N / ha, optimal 180 kg N / ha, super optimal 360 kg N / ha) were applied to whole plots. Plots were one row wide and 15.2 m long.

Samples were collected twice per week by taking one flower from the upper third of the plant canopy and one from the lower third on each of three plants per plot. Sampling was conducted from the onset to conclusion of flowering. The numbers of each species of flower thrips and the predatory bug, *Orius insidiosus*, were counted for each sample. The primary species of thrips encountered in the spring in northern Florida are *Frankliniella occidentalis, F. tritici, F. bispinosa* and *F. fusca.* Because it was not possible to identify thrips larvae to the species level, these were combined into a single group for analysis. The data were transformed to Ö(y + 0.375) and then subjected to analysis of variance.

Results: Two species of thrips, *F. occidentalis* and *F. tritici,* were the most common during the spring, with *F. fusca and F. bispinosa* rarely being found . The predator, *Orius insidiosus,* was also rarely encountered in tomato flowers. *F. occidentalis* was the predominant species of thrips in the early part of the season. Populations of *F. occidentalis* peaked during the first week of May, then rapidly decreased. Populations of *F. tritici* peaked approximately two weeks later but also rapidly declined thereafter. Significantly more adult thrips were found in the upper canopy flowers than in the lower canopy flowers. The opposite was true for immature thrips. Significantly more larvae occurred in the lower canopy flowers.



Mean numbers of thrips per flower from the upper and lower canopy of tomato plants grown during the spring 2000 season in Quincy, Florida. Significantly more adults of *Frankliniella occidentalis* (a) and *F. tritici* (b) were found in the upper flowers, while significantly more immature thrips (c) were found in the lower flowers.

CRIS 6615-22000-015-00D

EFFECTS OF INTER-PLANTING TOMATOES AND PEPPERS ON HERBIVORE-NATURAL ENEMY INTERACTIONS

S. Reitz, I. Baez [1] and J. Funderburk [2]

Objectives: Although tomatoes and peppers are both susceptible to thrips-vectored tomato spotted wilt virus, these plants differ in their suitability as hosts for thrips as well as for an important natural enemy of the thrips, *Orius insidiosus*. Tomatoes are a less suitable reproductive host than peppers, but adult thrips are highly mobile and will colonize less suitable reproductive hosts. Likewise, *O. insidiosus* rapidly track thrips. Therefore this research was designed to determine if populations of thrips and their natural enemies in tomatoes would be affected by the proximity to pepper plants, and if increasing nitrogen fertilization results in larger populations of flower thrips in tomatoes and peppers. If tomatoes planted closer to peppers have lower thrips populations, suitable host plants could be used to augment natural enemy populations for reducing pest populations in less suitable crop hosts.

Methods: Pepper and tomato plants were transplanted at the University of Florida North Florida Research and Education Center, in Quincy during July. Plants were grown on white plastic mulch for the fall season and drip irrigated. Each plot consisted of a row of 20 tomato plants, 20 pepper plants, and 20 tomato plants. To analyze the effect of nitrogen fertilization and proximity to the peppers had on populations of thrips and their natural enemies in tomatoes, the experiment was laid out as a split plot design with five replicate blocks. Three nitrogen fertilizer treatments (sub optimal, 90 kg N / ha; optimal, 180 kg N / ha; super optimal, 360 kg N / ha) were applied to whole plots, and the positions of tomato plants relative to pepper plants were the subplot treatment. Seven distance locations were established for tomatoes, and pepper plots were divided into five groups of four plants each for sampling. Comparisons were also made between the two types of host plants. On each sample date, five flowers from each location were collected. Sampling was conducted twice per week from the onset of flowering until the end of flowering.

Results: Because tomatoes begin flowering before peppers, availability of pepper flowers may have affected the early season populations. Significantly more thrips and *O. insidiosus* were found in pepper flowers than in tomato flowers. We did not find significant differences in thrips or *Orius* populations among tomatoes in relation to their distance from pepper plants. For the fall season, the predominant species of thrips found in both pepper and tomato flowers was *F. tritici*, with the next most abundant species being *F. bispinosa*. *Frankliniella occidentalis,* the western flower thrips, and *F. fusca* were rarely encountered. The nitrogen fertilization level did not have a significant impact on the number of adults; however greater numbers of immatures were found in the optimal and super optimal nitrogen levels.

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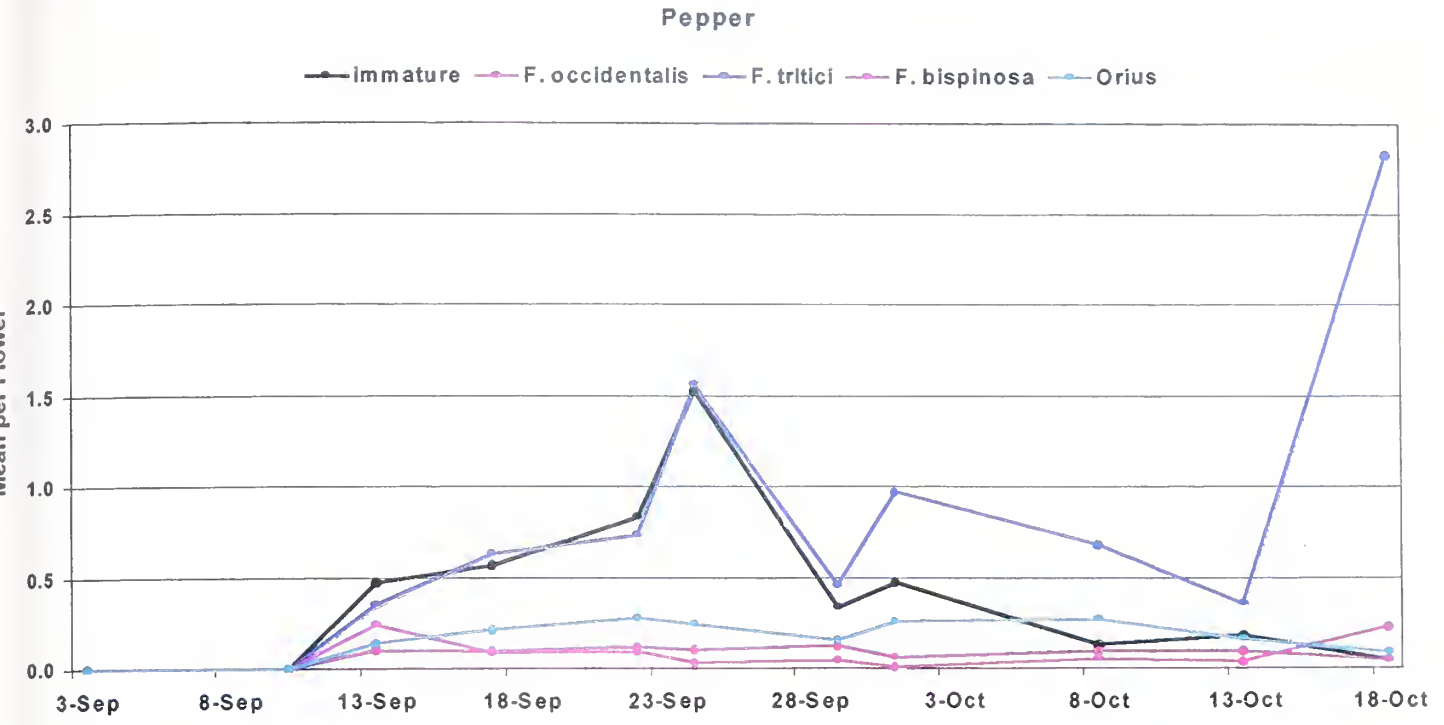
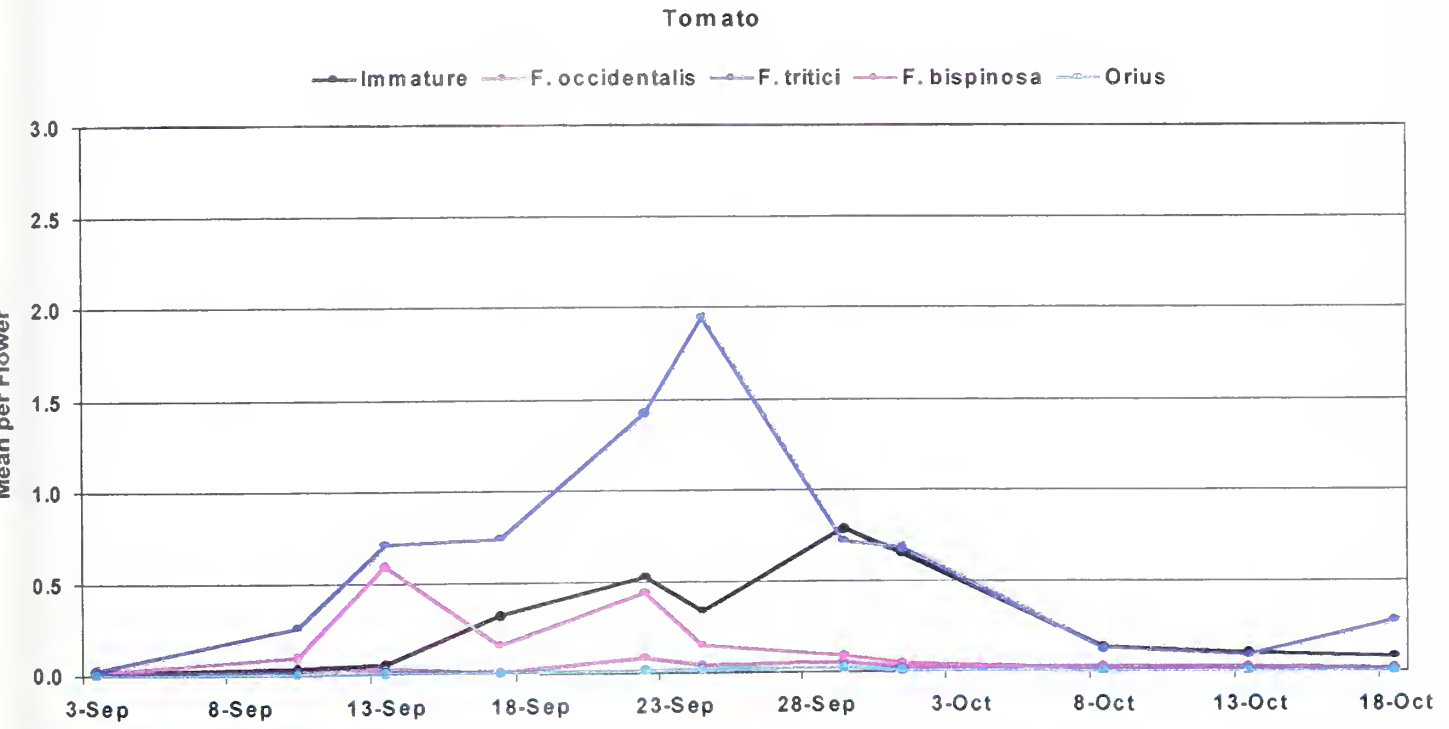
[1] Florida A&M University and USDA-ARS

[2] University of Florida





Mean number of thrips and *Orius insidiosus* per flower found during the fall 1999 season. *Frankliniella tritici* was the predominant thrips species found in both types of plants. Significantly more thrips and *Orius* were found in pepper. The position of tomatoes in relation to peppers had no significant impact on thrips or *Orius* populations.

CRIS 6615-22000-016-00D

DEVELOPMENT OF A POLYUBIQUITIN-REGULATED RED FLUORESCENT PROTEIN MARKER FOR TRANSGENIC INSECTS

A.M. Handler and R.A. Harrell II

Objective: To develop a new visible marking system that can be used for selecting transgenic insects and detecting them after field release

Methods: The DsRed fluorescent protein gene from the sea anemone, *Discosoma striata*, was tested as a marker in transgenic insects by placing it under polyubiquitin promoter regulation and integrating it into the *Drosophila melanogaster* genome by *piggyBac* transformation. The pB[PUb-DsRed1] vector was coinjected with phspBac helper into 713 embryos, from which 305 G_0 larvae hatched with 191 surviving to adulthood. The G_0 adults were backcrossed in 81 small groups with G_1 larvae and pupae screened for DsRed fluorescence. Fluorescent insects were observed in 26 of the lines which was verified by detailed inspection of all emerging G_1 adults, yielding an approximate transformation frequency of 25%. DsRed and the enhanced green fluorescent protein (EGFP) were observed at all developmental stages under a Leica MZ-12 stereozoom fluorescent microscope using a mercury lamp and ultraviolet light filter sets for DsRed (Texas Red) and EGFP (FITC). Digital images were obtained with a SPOT-1 cooled CCD camera and processed with Adobe Photoshop 4.0 software.

Results: *Drosophila* transformed with a *piggyBac* vector marked with polyubiquitin-regulated DsRed were easily detected by red fluorescense as larvae, pupae and adults. Fluorescense throughout development and in most tissues was verified by examination of individual transgenic flies, which is consistent with the normal activity of the polyubiquitin promoter. DsRed expression was relatively bright and more intense as compared to *Drosophila* transformed with an EGFP marker regulated by the same promoter. Detection of DsRed should be less susceptible to autofluorescense since it emits outside the range of most types of natural fluorescense, and we verified this by comparing fluorescence from *Drosophila* transformed with DsRed, EGFP, and non-transformed pupae under the filter sets used for EGFP (FITC) and DsRed (Texas Red). Under the FITC filter the EGFP transformed pupa appeared bright green, the DsRed pupa was pink-orange, and the non-transformed pupa emitted easily detectable yellow autofluoresence from its pupal case. In contrast, under the Texas Red filter, the DsRed pupa was bright red, with only barely detectable fluorescence from the EGFP and non-transformed pupae. The relatively high intensity of polyubiquitin-regulated DsRed in transformant *Drosophila*, and the lack of autofluorescenece under the Texas Red filter set is highly encouraging for use of the DsRed marker for transformant selection in many insects, and especially when double-markers are used requiring two fluorescent proteins. It should be especially useful for field detection of released transgenic flies.

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TRANSFORMATION OF THE ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS*, WITH THE *PIGGYBAC* TRANSPOSON VECTOR

A.M. Handler and S.D. McCombs[1]

Objective: To test the ability of the *Trichoplusia ni piggyBac* transposon vector to mediate germline transformation in a tephritid fruit fly.

Methods: The *T. ni piggyBac* transposon vector was previously shown to mediate efficient gene transfer in the Mediterranean and Caribbean fruit flies, and we attempted to extend this technology to another tephritid species, the Oriental fruit fly, using the medfly *white*$^{+}$ marker to detect trasngenic flies. In two experiments, 3,742 embryos from a *white eye* strain were injected with vector and helper plasmids at concentrations of 500 µg/ml and 300 µg/ml, respectively. From these injections, 243 G_0 embryos survived to adulthood and were individually backcrossed to the host strain. Of these, 157 were fertile with three of the G_0 matings yielding progeny with pigmented eyes. One of the lines, Bd1-61, yielded 119 putative transformants having red-orange eye color phenotypes, a second line, Bd1-115, yielded five G_1 offspring with yellow eyes and a third line, Bd1-137, yielded nine offspring with pale pink eyes. Putative transformant G_1 offspring were backcrossed individually, and their G_2 transformant offspring and subsequent generations were intermated. Vector integrations were verified by Southern DNA hybridization and *piggyBac* elements within the *B. dorsalis* genome were isolated and sequenced after PCR gene amplification.

Results: The *piggyBac* gene transfer vector system was shown to be effective in another economically important tephritid fruit fly species by generating three transformant lines. However, Southern DNA hybridization detected the presence of genetic elements in the *B. dorsalis* genome that were closely related to the *piggyBac* vector originally isolated in the *Trichoplusia ni*. Hybridization patterns indicated a range of 10-20 elements per genome in several wild and mutant strains, with intact (or normal size) and defective elements. Several of these elements were isolated by PCR gene amplification from several strains and their sequencing indicated greater than 95% sequence identity among them. Given *piggyBac*'s range of function, and compared to similarly active transposon vectors, the existence of *piggyBac* in distantly related species is not surprising, and it is likely to have occurred by horizontal transmission. The apparent discontinuity of *piggyBac*'s presence is unusual, and more complete searches are in progress. The existence of *piggyBac*, or related transposons in various insect species has important implications for *piggyBac* transgene stability and horizontal transmission, and the use of *piggyBac* transgenic strains for field applications may require strategies to ensure vector stability.

[1] Collaborator from the University of Hawaii and USDA-APHIS-PPQ, Waimanalo, Hawaii

CRIS 6615-22000-016-00D

LAMBSQUARTERS IN A MAIZE AGROECOSYSTEM: A POTENTIAL REFUGE FOR NATURAL ENEMIES OF THE FALL ARMYWORM

D.L. Johanowicz [1], H.A. Smith [1], A. Sourakov [1] and E.R. Mitchell

Objective: Generalist natural enemies may be important in regulating pest populations in annual crops in part because they may be able to survive temporary absence or scarcity of a target pest by attacking alternate hosts. Many species of generalist parasitoids of the fall armyworm (FAW) can be found in maize agroecosystems throughout the growing season. However, natural levels of control do not usually keep FAW populations under economic levels, and efforts to augment, conserve, and/or manipulate FAW populations are usually not adequate.

Habitat management to enhance populations of generalist beneficial arthropods may be useful in a biological control program to manage the FAW. By ensuring a supply of supplementary foods or alternative hosts, natural enemy populations may build up earlier in the season for subsequent availability when target pest populations begin to increase. We have observed that lambsquarters host a variety of noctuid larvae. Wealso have noted the presence of adult parasitoids and predators searching in the infested lambsquarters. The purpose of this study was to determine the species composition and abundance of noctuid larvae and their natural enemies on lambsquarters in a north Florida maize field in efforts to evaluate this plant's potential as a nursery crop for enhancement of FAW biological control.

Methods: The study site was planted to maize (field corn), located in Putnam County, FL. Lambsquarters grew abundantly along the irrigation ditches which ran the length of the field every 16th row. We sampled ten consecutive plants at each of 12 sample sites, repeated every week for five weeks. In the laboratory, plants were inspected for larvae and beneficials and the data were recorded and tabulated. Noctuid larvae were dissected to determine levels of parasitism and species of parasitoid. Other adult beneficials were noted as present in a sample (e.g. *Orius insidious*) but were not tabulated because the sampling method used was not appropriate to estimate populations of mobile predators and adult parasitoids.

Results: High numbers of southern armyworm, a species which is not a serious pest of maize, were present in the lambsquarters, whereas very few FAW, a serious pest of maize, were found in these weeds. Two other noctuids, the beet armyworm and the cabbage looper were found in moderate numbers. High levels of parasitism by generalist parasitoids occurred in all of the noctuids sampled in the first three weeks while larval densities were high, averaging between 35 - 40 % for all species combined. The dominant parasitoid was *Cotesia marginiventris* (Hymenoptera: Braconidae) ; other parasitoid species present in low numbers were *Meteorus autographae* (Hymenoptera: Braconidae) and *Chetogena scutellaris* (Diptera: Tachinidae). Many predators (predominantly Orius insidiosis) were present on the lambsquarters throughout the sampling period. These data indicate that lambsquarters in the maize agroecosystem may act as a refuge for parasitoids and predators, potentially providing a reservoir of natural enemies for enhanced biological control of the fall armyworm.

[1] University of Florida

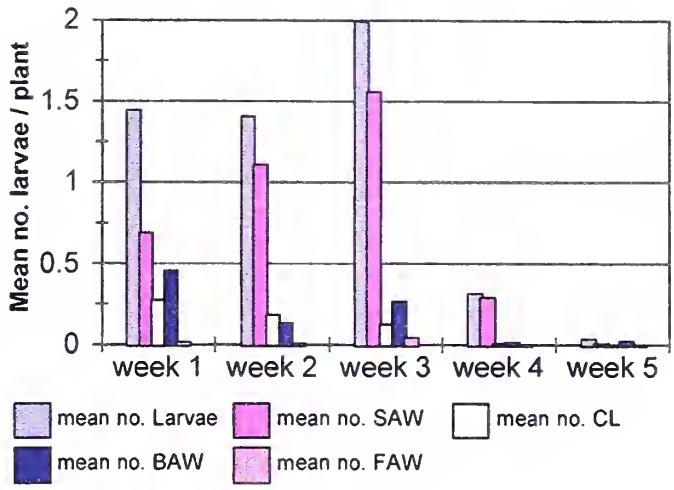


Fig. 1. Abundance (mean no. larvae/plant) of noctuid larvae on lambsquarters located along irrigation ditches in a North Florida maize field (field corn). Ten plants were sampled per site; a total of 12 sites were samples each week.

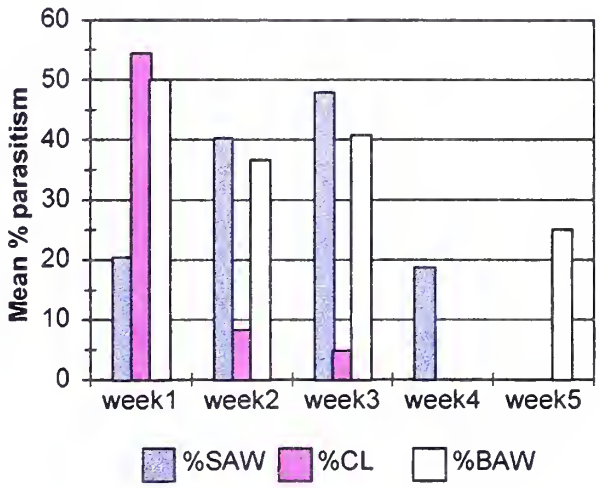


Figure 2. Parasitism levels of noctuid larvae found on lambsquarters. Parasitism was determined by dissected collected larvae in the laboratory. *Cotesia marginiventris* was the dominant parasitoid.

CRIS 6615-22000-016-00D

CONTROL OF MALE FALL ARMYWORM MOTHS USING AN ATTRACT AND KILL STRATEGY

R.L. Meagher, Jr.

Objective. Techniques are currently being utilized to modify lepidopteran pest populations using adult behavioral methods. Commercially-established techniques utilize an attract and kill strategy where male moths are attracted to a matrix composed of a combination of pheromone plus toxicant. However, for the strategy to be successful, the matrix must be applied to plants, moths must come in contact with the matrix, and relatively high doses of the toxicant are necessary. This report documents the first of a series of experiments to design an attract and kill strategy in which male moths are attracted to and feed on a toxicant solution.

Methods. Previous research with other noctuid moths has shown that the addition of a stimulant, usually a sugar solution, increases feeding from insecticidal solutions. This first experiment was designed to determine the balance between sugar amount and toxicant dose needed to produce the highest mortality. Male fall armyworm moths (*Spodoptera frugiperda*) were placed in 24-cm square screen cages under natural lighting conditions. Moths (< 24 h old) were fed solutions composed of different sucrose concentrations and Permethrin doses that varied from 0 to 0.15%. Five levels of each variable yielded 25 separate treatments. Each screen cage was a replication, and each treatment contained 4 replications. The number of moths in a cage varied from 10-40, with an average of 20 moths per cage. Mortality was determined 24 h later.

Results. Moth mortality was influenced by both Permethrin dose and sucrose concentration ($P < 0.02$) (Table 1). The interaction between both variables was not significant ($P > 0.3$). Differences in mortality among Permethrin doses was attributed to very low control mortality (< 1%), as there was no difference in mortality among the other doses. More moths apparently fed and were killed on solutions that had a ≥10% sucrose concentration.

Table 1. Fall armyworm male moth mortality when fed solutions composed of different Permethrin doses poured into varying sucrose concentrations in small screen cage bioassays.

Permethrin Dose (%)	Percent Mortality	Sucrose Concentration (%)	Percent Mortality
0.05	79.1 ± 3.0 a	10	67.7 ± 8.2 a
0.075	78.7 ± 3.5 a	20	64.6 ± 8.0 ab
0.025	78.3 ± 3.7 a	30	63.2 ± 9.0 abc
0.15	74.9 ± 4.0 a	5	57.8 ± 7.7 bc
0	0.9 ± 0.06 b	0	54.1 ± 7.1 c

CRIS 6615-22000-016-00D

EFFECT OF SWEET ALYSSUM PLANTS IN CABBAGE ON PARASITISM OF BEET ARMYWORM BY *COTESIA MARGINIVENTRIS*

E.R. Mitchell and D.L. Johanowicz[1]

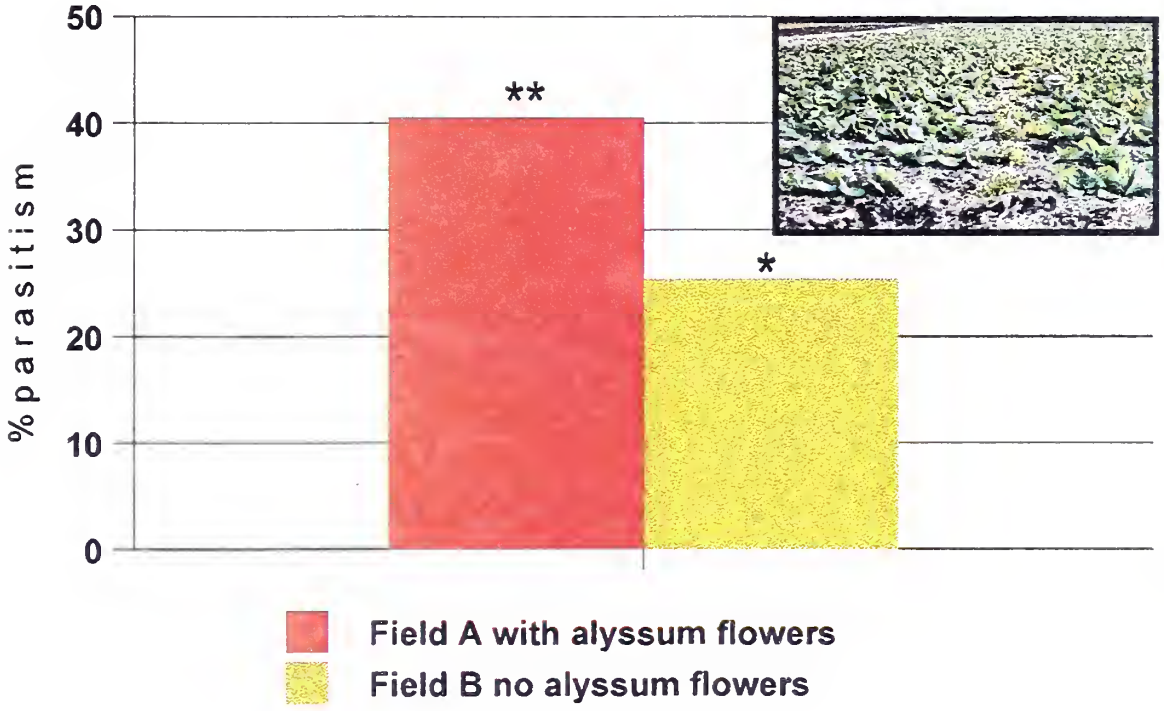
Objective: Access to sweet alyssum flowers under glasshouse conditions increased survival of *Cotesia marginiventris* parasitoids 4.8 times longer than parasitoids provided water only. The present study was conducted to determine if planting this hearty winter annual in cabbage would result in increased parasitism of beet armyworm (BAW) larvae by *C. marginiventris*.

Methods: Young, blooming sweet alyssum (Sa) plants were transplanted in a 20 ac cabbage field (Field A) on 12 January 2000. A nearby 20 ac field (B) without Sa plants served as the control. Fifteen Sa plants were transplanted across rows (1/row, Fig. 1) at 10 locations equally spaced around the periphery of Field A. Three sites were located along the 2 longest sides of the field and 2 sites along the 2 shorter sides. The Sa plots were located ca 25 ft into the field from the margins. No *C. marginiventris* parasitoids were released in either field. Sentinel BAW larvae were allowed to feed on collard leaves for 24-48 h prior to being placed on cabbage plants in each field. Plants bearing sentinel larvae were located at 9 pre-determined locations throughout Fields A and B. Larvae were set out on a weekly schedule beginning 12 January and continued through 14 March. Larvae were collected from the sentinel cabbage plants on a weekly basis beginning 19 January and continuing through 21 March. The larvae were returned to the laboratory and dissected to determine the level of parasitism. Percentage parasitism was averaged over all locations in each field on a weekly basis. For analysis, percentages were transformed to $\sqrt{n} + 0.5$ and subject to a *t*-test with weeks serving as replicates.

Results: The number of BAW larvae recovered from sentinel cabbage plants frequently were low due to persistent spraying of insecticides (almost exclusively Bt's) by the grower cooperator. Nevertheless, parasitism of BAW larvae by *C. marginiventris* in the field with Sa plants (Field A) was significantly greater ($P<0.05$, paired *t*-test) than in the control field (B) with no Sa plants (Fig. 1). Parasitism of BAW larvae in both fields was due entirely to naturally occurring *C. marginiventris* populations thus confirming the presence and active reproductive status of this parasitoid during the winter months in Northeast Florida. The results also demonstrate the beneficial effects of flowering plants, in this case Sa, as a food source to boost the effectiveness of *C. marginiventris* under actual field conditions. The increased parasitism of sentinel BAW larvae by *C. marginiventris* noted in the field with Sa plants possibly was due to attraction of *C. marginiventris* parasitoids into the field from outside sources and their increased survival over a longer period due to the presence of a nectar source provide by Sa flowers.

[1] University of Florida

Fig 1. Parasitism of Beet Armyworm larvae by Cotesia marginiventris.
(Stars indicate significance between means, P<0.05).



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EFFECT OF SWEET ALYSSUM PLANTS IN CABBAGE ON PARASITISM OF DIAMONDBACK MOTH BY *DIADEGMA INSULARE*

E.R. Mitchell and D.L. Johanowicz[1]

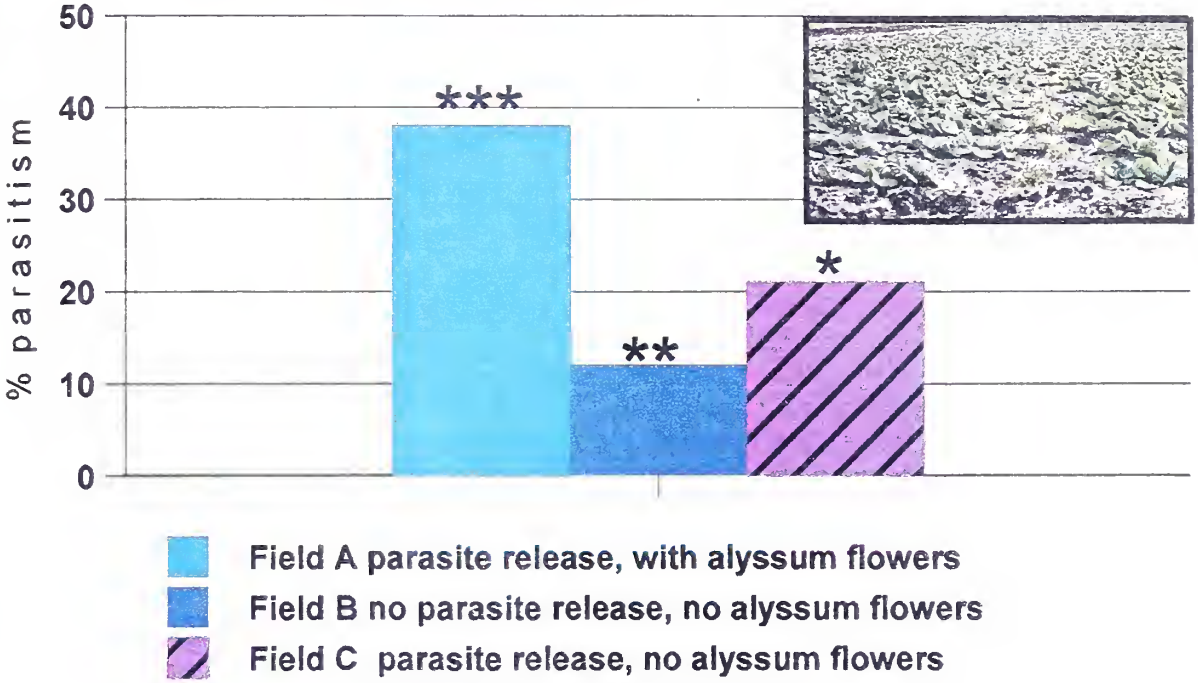
Objective: Access to sweet alyssum flowers under glasshouse conditions increased survival of *Diadegma insulare* parasitoids 12.7 times longer than parasitoids provided water only. The present study was conducted to determine if planting this hearty winter annual in cabbage would result in increased parasitism of diamondback moth (DBM) larvae by *D. insulare*.

Methods: Young, blooming sweet alyssum (Sa) plants were transplanted in a 20 ac cabbage field (Field A) on 12 January 2000. Two fields were used as controls, Field B (20 ac) and Field C (5 ac), neither of which had Sa plants. Fields A and B were ca. 0.25 mi apart and Field C was ca.1.5 mi from these fields. Fifteen Sa plants were transplanted across rows (1/row, Fig. 1) at 10 locations equally spaced around the periphery of Field A. Three sites were located along the 2 longest sides of the field and 2 sites along the 2 shorter sides. The Sa plots were located ca 25 ft into the field from the margins. Fifty pairs of *D. insulare* wasps were caged on each of 10 potted Sa plants for preconditioning 24 h before release adjacent to the Sa plots (1 cage/plot) on 21 January 2000. A second release of 50 pairs/plot was made on 23 February. A similar number of pairs of wasps (50/ac), preconditioned as for Field A, were released on 21 January and 23 February near the center of ea. quadrant in Field C (i.e., 32 pairs at ea of the 4 sites). Field B had neither Sa plants nor releases of *D. insulare* wasps. Sentinel DBM larvae were allowed to feed on collard leaves for 24-48 h prior to being placed on cabbage plants in each field. Plants bearing sentinel larvae were located at 9 pre-determined locations throughout Fields A and B and at 6 pre-determined locations in Field C. The sentinel larval sites were located well away from the wasp release sites. Larvae were set out weekly beginning 12 January and continued through 14 March. Larvae were collected from the sentinel cabbage plants on a weekly basis beginning 19 January and continuing through 21 March. The larvae were returned to the laboratory and dissected to determine parasitism. Percentage parasitism was averaged over all locations in each field on a weekly basis. For analysis, percentages were transformed to $\sqrt{n+0.5}$ and subject to ANOVA with fields as main effects (treatments) and weeks as replicates.

Results: The number of DBM larvae recovered from sentinel cabbage plants frequently were low due to persistent spraying of insecticides (almost exclusively Bt's) by the grower cooperator. Nonetheless, the results clearly showed a higher level of parasitism of DBM in Field A where *D. insulare* were released near Sa alyssum plots (Fig. 1) than in Field C (parasitoid releases only) or the untreated control (Field B). These results demonstrate the beneficial effects of sweet alyssum as a nectar food source for *D. insulare* which resulted in ca. a 2-fold increase in the level of parasitism of DBM compared to the release of parasitoids only (Field C) and ca. a 3-fold increase over DBM parasitism by naturally occurring populations of *D. insulare* (Field B). (Differences in mean parasitism significant at $P<0.05$, SNK test).

[1] University of Florida

Fig. 1. Parasitism of Diamondback Moth in cabbage.
(Stars indicate significant difference between means, P<0.05).



CRIS 6615-22000-016-00D

ECOLOGY AND BEHAVIOR OF TEPHRITID FRUIT FLY PARASITOIDS IN MEXICO AND FLORIDA

J. Sivinski and M. Aluja[1]

Objective: Biological control sometimes has a major affect on pest fruit fly populations, but in other instances parasitoids either fail to become established or do not flourish and are only rarely recovered. One reason for the failures may be that the wrong parasitoids for the local conditions are being used in control efforts. Parasitoids, including those that attack tephritids, are often specialize, being active in certain trees, locations, seasons, and times of day. Information on when, where, and how natural enemies hunt for pest fruit flies may help predict which species should be introduced, when they should be periodically released in large numbers (augmented) in particular areas, or how they can be conserved and enhanced through manipulation of the environment.

Methods: In Veracruz State, Mexico the spatial and temporal distributions of braconid, eulophid, eurytomid, eucoilid, and diapriid parasitoids attacking five species of *Anastrepha* fruit flies have been studied for seven years. Field samples have been taken to determine altitudinal and regional patterns of abundance. In the laboratory, tests have been used to examine what factors in the environment parasitoids use to locate hosts, and how different species exploit a shared resource.

Results: Regional sampling discovered that the abundances and host ranges of various parasitoid species change markedly in different environments. Several species that have been considered as candidates for augmentative release, particularly *Doryctobracon areolatus* and *D. crawfordi,* were found to have distinct altitudinal (temperature and moisture) preferences. This will aid future efforts to maintain fly free zones through mass-rearing parasitoids. Further support was obtained for the hypothesis that host fruit density and diversity is an important factor in the survival and increase of certain parasitoids. Since most parasitoids are not as capable of dispersal as their fruit fly hosts, a variety of trees, fruiting at different times, may provide the best conditions for parasitoids to become established and increase in numbers. A long-experiment where native fruit tree diversity will be re-established near agricultural areas is underway. These trees will harbor nonpest fruit flies that are attacked by the same parasitoids as the pest species. It is predicted that parasitism will be enhanced and that the resulting decline in infestation will improve the diets and economies of rural people. Foraging by pupal parasitoids was investigated with the goal of identifying the most efficient pupal parasitoid for mass-release. The diapriid *Coptera haywardi* was best able to attack fruit fly pupae at various depths in different types of soil. Males of several genera of fruit-fly attacking braconids appear to produce a pheromone. Male volatiles have been collected from two species and further collections are planned for a number of native and exotic species. At present, there are no effective traps to estimate the population density of these parasitoids, and the search for attractants is the first step in their development. Rearing techniques were perfected for the braconid *Utetes anastrephae*. This is a locally important parasitoid throughout the New World, but investigations into its biology had been hampered by an inability to maintain it in the laboratory.

[1]Instituto de Ecologia, Xalapa, Veracruz, Mexico

CRIS 6615-22000-016-00D

BIOLOGICAL CONTROL OF MEDFLY IN GUATEMALA

J. Sivinski, T. Holler[1], and P. Rendon[2]

Objectives: The Mediterranean fruit fly is abundant in Central America and threatens to move northward into Mexico and ultimately the US. It is prevented from doing so by a sterile fly and pesticide barrier maintained on the Mexican-Guatemalan border by the international organization MOSACMED. In recent years this barrier has become increasingly permeable and new techniques are sought to improve it and make it more environmentally benign. Biological control has two possible roles in the region:1) Large numbers of mass-reared parasitoids can be combined with sterile fly releases, and 2) new parasitoids can be introduced that will result in fewer adult flies and contribute to integrated pest management.

Methods: *Augmented release of parasitoids-* The ability of *Diachasimorpha tryoni, D. kruasii,* and *D. longicaudata* to attack medfly, singly and in combination was examined in field cages placed over coffee in a mountainous region of southern Guatemala. Competition among the species will reveal which is the most suitable species for the area. In order to facilitate the aerial release of parasitoids, tests are being made of the effects of chilling (a prerequisite for aerial release) on the fecundity and longevity of the 3 *Diachasmimorpha* species and another braconid *Fopius arisanus.* A pupal parasitoid, *Coptera haywardi*, is being considered for augmentative release. Field cage studies in coffee plantations were carried out to determine its ability to survive and attack medfly hosts under Guatemalan conditions. *Parasitoid introduction-* Explorations for more effective parasitoids for use both in establishment attempts and augmented releases are ongoing in Mexico and Kenya. The later collections are part of a collaborative effort with the Universities of Hawaii, Florida and Texas A&M, and the International Center for Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). Candidate parasitoids are colonized at USDA-APHIS facilities in Guatemala.

Results: *Augmented releases-* Field cage tests found little competition among *Diachasmimorpha* spp., and mixed species releases are unlikely to have any deleterious effects. Chilling, such as encountered in automated aerial release devises, had little effect on the fecundity or longevity of any of the species. In field cages placed over coffee, *C. haywardi* was able to locate and attack pupae at soil depths up to 15 mm. Even unsuccessful attacks by *C. haywardi* were found to cause substantial mortality, and the species may be twice as effective as previously believed. A mass-rearing scheme that first exposes hosts to a larval parasitoid and afterwards to a *C. haywardi* doubled natural enemy production. When perfected this technique may lower the costs of parasitoid mass rearing. *Parasitoid introduction-* A new African species, *Psyttalia concolor*, was found to attack medfly under semi-natural conditions in field cages. A new quarantine facility was constructed in Guatemala and approved by APHIS. The first shipment of 2 species of *Fopius* collected from medfly in Kenya arrived at the quarantine and colonization efforts are proceeding. These parasitoids, (*F. caudatus* is an egg-pupal parasitoid and *F. ceratitivorus* is a parasitoid of 1st instar larvae) attack the very early and vulnerable stages of fruit fly hosts and are potentially very useful in biological control. Funding was obtained to continue the African collections and more shipments to Guatemala are planned for the following year.

[1]USDA-APHIS, Gainesville, FL
[2]USDA-APHIS, Guatemala City, Guatemala

CRIS 6615-22000-016-00D

EVALUATION OF INSECTARY CROPS TO INCREASE NATURAL BIOLOGICAL CONTROL ON SMALL FARMS AND ORGANIC FARMS

H.A. Smith[1] and E.R. Mitchell

Objective: 1) To evaluate the potential of a variety of flowering plants to attract and maintain populations of beneficial insects in the field and 2) to examine the effect of roselle (*Hibiscus sabdariffa*) on the longevity of a minute pirate bug (*Orius insidiosus*) and a ladybird beetle (*Coleomagilla maculata fuscilabris*).

Methods: 1) In May 2000, Cosmos (*Cosmos bipinnatus*) and yarrow (*Achilleae millefolium*) were tested as insectary crops in two separate studies in a commercial corn field near Bunnell, Flagler County, FL. Insectary plants were intercropped every 0.3 m with field corn in small (4 x 5 m) plots and arranged in a randomized complete block design. Plants were sampled weekly using a vacuum sampler for beneficial insects, which were identified to family. Damage ratings were taken for intercropped and control corn. Corn seedlings infested with fall armyworm larvae were placed in pots among intercropped and control corn for 48 h. Exposed larvae ("sentinel larvae") were then observed in the laboratory to gather information on percent parasitism.

2) A stand of *Spermacoce verticillata*, an annual weed common in Florida, was transplanted on the grounds of the USDA in Gainesville in April and sampled for beneficial insects using a vacuum sampler during the summer months.

3) A hedgerow of roselle was planted on the USDA grounds to be evaluated as a potential insectary crop. Roselle has extra-floral nectaries and has been associated with high numbers of beneficial insects in other studies. A greenhouse study evaluating the behavior and longevity of *Orius insidiosus* and *Coleomagilla maculata fuscilabris* on young roselle plants was initiated in August.

Results: 1) Damage ratings and percent parasitism on field corn were not affected by the presence Cosmos or yarrow. Beneficial insects were found in association with the cosmos; yarrow did not flower within the time frame of the study and did not attract beneficials. Both cosmos and yarrow require a great deal of management, and like most annual flowering plants, may not be grown on a large scale by resource-limited farmers primarily for the purpose of maintaining stable populations of beneficial insects. Organic growers revealed in interviews during the spring that they were interested in plants that could help maintain stable populations of beneficial insects, but that these insectary crops would have to be low-maintenance. 2) *Spermacoce verticillata* proved to be a promising insectary crop in that it required little maintenance, and unlike many flowering plants, bore flowers throughout the summer, providing resources to a range parasitic and predatory Hymenoptera. These observations are consistent with earlier studies on *S. verticillata* as a resource for *Larra bicolor*, a mole cricket parasitoid. Roselle also grew well throughout the summer with little maintenance, although few beneficials were observed on extra-floral nectaries until plants were about 3 months old. The greenhouse study is on-going.

[1] University of Florida

Table 1. Families of parasitic or predatory Hymenoptera collected from *Spermacoce verticillata* or *Cosmos bipinnatus*.

Spermacoce verticillata	*Cosmos bipinnatus*
Braconidae	Bethylidae
Chalcididae	Braconidae
Eucoilidae	Encyrtidae
Eulophidae	Eucoilidae
Pteromalidae	Eulophidae
Scelionidae	Mymaridae
Sphecidae	Pteromalidae
Tiphiidae	Scelionidae
Trichogrammatidae	Trichogrammatidae

CRIS 6615-22000-016-00D

HOST AGE AND SPECIES PREFERENCE IN *METEORUS AUTOGRAPHAE* (HYMENOPTERA: ICHNEUMONIDAE)

A. Sourakov[1] and E.R. Mitchell

Objective: In the present study, we offered larvae of two hosts - beet armyworm (BAW) and cabbage looper (CL) - to females of *M. autographae* in a large outdoor cage. The objective was to determine which, if either, of the host species is favored by *M. autographae* for oviposition and which larval stage might be preferred under close-to-natural conditions. The study was conducted in December 1999-January 2000 in Gainesville, FL, during the time when cole crops such as cabbage and collard are grown and when the cabbage looper often becomes a pest of these crops.

Materials and Methods: To determine host size and species preference, two-to-six d old females were released in an outdoor 1.5x1.5x3.0-meter cage. Potted mature collard plants were placed inside the cage and infested with CL and BAW larvae. Two blooming sweet alyssum plants were provided as a nectar source. Host larvae were collected 48-72 h later and dissected for parasitoid eggs. In the 1st treatment, only BAW was used as a host. In the 2nd treatment, BAW and CL were placed together on one of the three collard plants. The other two plants were infested with CL only: one with early 2nd instar larvae, another with 3rd instar. A mixture of BAW and CL was used in the 3rd treatment, but this time one of the two collard plants was infested with late 2nd instar larvae that developed to the 3rd instar during the treatment; the other plant was infested with 3rd instar larvae which matured to the 4th instar. In the 4th experiment, we released *M. autographae* females in the cage in which the collard plants were infested with mature CL larvae.

Results: Dissections suggest that *M. autographae* favored 3rd instar larvae for oviposition. In the second treatment, BAW were favored for oviposition when they were mixed with CL, but when CL only were present on a plant, total parasitism was higher. Though 70% of CL larvae were parasitized, a single egg was found in all younger larvae, whereas 56% of older CL larvae received more than one egg. Only a single parasitoid survives per host (first-to-emerge parasitoid larva kills its siblings), and superparasitism is wasteful; perhaps, superparasitoidism would have been reduced or not occurred if adult wasps were free to disperse. Results of the 3rd treatment imply that smaller larvae were favored, with CL being a more favored host. Among 150 larvae collected from the 4th treatment, only 11% yielded parasitoid cocoons.

Our observations suggest that given a choice, *M. autographae* favor late 2nd-3rd instar larvae for oviposition whereas older larvae are less vulnerable to parasitism. Both CL and BAW are acceptable hosts at these stages, and probably would be attacked by *M. autographae* in the field. However, our experiments suggest that parasitism will be relatively low, and therefore *M. autographae* alone cannot be expected to be an effective pest control agent. Instead, the species should be viewed in the context of a total IPM program involving parasitoids and other biological control measures. Superparasitism by *M. autographae* shows that the more favored host species and stage may receive more than one egg, at least under cage conditions. This and the fact that relatively few of the larger larvae were parasitized in the 1st and 4th experiments suggests that with age, the larvae might develop some defensive mechanism (chemical, behavioral, or/and physiological) against parasitism.

[1]University of Florida

CRIS 6615-22000-016-00D

SEX ALLOCATION IN PROGENY OF *DIADEGMA INSULARE* (HYMENOPTERA: ICHNEUMONIDAE)

A. Sourakov[1] and E.R. Mitchell

Objective: *D. insulare* is an important parasitoid of diamondback moth, *Plutella xylostella*. However, it is not currently available commercially to growers, because its production can be labor consuming and therefore expensive. For augmentative releases, it is advantageous to produce as many parasitoids as possible in relation to the number of available hosts with the highest possible female to male ratio. The sex ratio of lab-produced *D. insulare* varies greatly depending on the multitude of factors. The objective of this study was to determine how the length of oviposition correlates with the numbers and the sex ratio of the resulting progeny.

Materials and Methods: Sixteen females of *D. insulare* were each offered 100+ diamondback larvae in one-gallon containers; a streak of honey was placed in every container and larvae were supplied with collard leaves. Every 24 hours, females were transferred into a new container with a fresh batch of larvae. The previously exposed larvae then were held for parasitoid emergence. Sex ratios and numbers of the resulting progeny were assessed for each of the 24-h oviposition periods.

Results: As shown in Fig. 1 and 2, the total number as well as the percentage of females in progeny increased during the first four days of oviposition. These observations suggest that for mass-rearing of *D. insulare*, techniques allowing prolonged oviposition should be considered to improve the sex ratio of the progeny.

[1] University of Florida

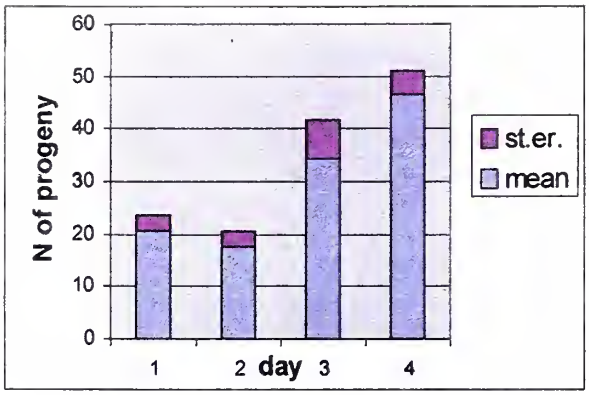


Fig. 1. Increase in number of progeny resulting from four consecutive days of oviposition by a single *D. insulare* female.

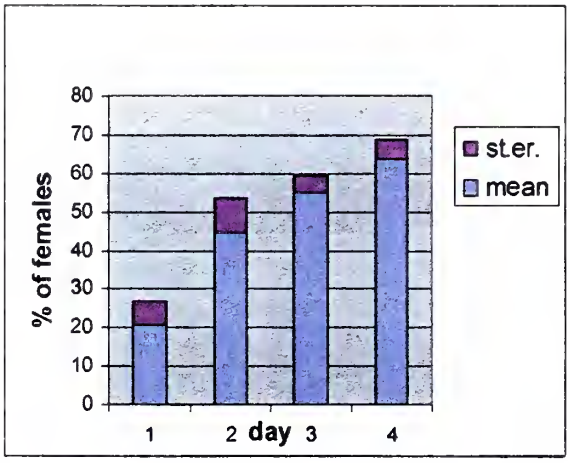


Fig. 2. Increase in the rate of females in progeny resulting from four consecutive days of oviposition by a *D. insulare* female.

CRIS 6615-22000-016-00D

SEX ALLOCATION OF PROGENY BY *METEORUS AUTOGRAPHAE* (HYMENOPTERA: ICHNEUMONIDAE)

A. Sourakov[1] and E.R. Mitchell

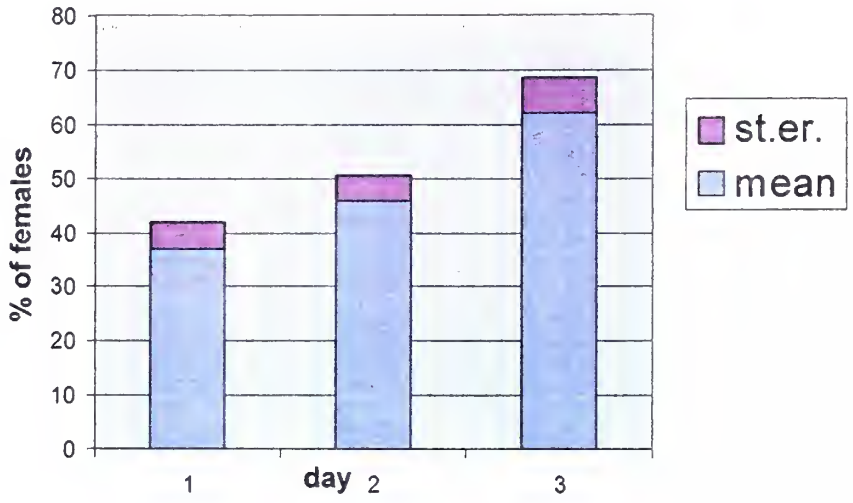
Objective: The parasitoid *Meteorus autographae* Muesebeck was studied extensively as an important control agent of noctuid pests, such as soybean looper (*Pseudoplusia includens* Walker). In the laboratory, females attack larvae of 1st through 5th instar of a wide variety of hosts. It is known that females can control the sex of the egg. Several species were shown to lay male eggs first during their life. While rearing *M. autographae* for host-choice experiments, we noted that the sex ratio of the progeny often was skewed toward males. It is important for mass-rearing of a parasitoid to obtain a female-biased sex ratio. Thus, our objective was to evaluate how sex ratio changes with time when *M. autographae* females are allowed to oviposit continuously for several days.

Materials and Methods: Fifteen females of *M. autographae* were each offered 30 beet armyworm larvae in a Petri dish; a streak of honey was placed in every dish. Every 24 h females were transferred into a new Petri dish with a fresh batch of larvae. The previously exposed larvae then were transferred to artificial diet and held for parasitoid emergence. Sex ratios of the resulting progeny were assessed for each of the 24-h oviposition periods. They were compared, using the table of one-tailed probabilities for the binomial test.

Results: As shown in Fig. 1, the number of female progeny increased during the first three days of oviposition. On the second day of oviposition, the number of females increased to an average of 45% from 36% of the first day. On the third day, the percentage of females increased once again to an average of 62%. These increases are statistically significant, with P=0.029 and P=0.018, respectively. These observations suggest that for mass-rearing of *M. autographae,* techniques allowing prolonged oviposition should be considered to improve the sex ratio of the progeny.

[1] University of Florida

Fig. 1. Sex ratio in the progeny of *Meteorus autographae* during the first three days of oviposition.



CHEMISTRY

CRIS - 6615-22000-012-00D--Chemistry and Biochemistry of Insect Behavior, Physiology, and Ecology

CRIS - 6615-22000-012-10R–Mechanism of Detection of Chemical Signals by Parasitic Wasps

CRIS - 6615-22000-012-12S–Field Tests of Tritrophic Plant-Insect Interactions

CRIS 6615-22000-012-00D

VOLATILES EMITTED BY SCLEROTIUM ROLFSII CULTURES AND INFECTED PEANUT PLANTS

Y.J. Cardoza and J.H. Tumlinson

Objective: To investigate the emission of volatile compounds from cultures of the peanut white mold fungus, Sclerotium rolfsii, and from peanut, Arachis hypogea, plants infected with this pathogen.

Methods: A) Peanut white mold fungus was cultured in potato dextrose agar (PDA) plates by placing a sclerotium, compacted mycelia, in the center of the plate. Volatiles emissions from three 5-d old culture plates were collected for 2 h. Samples were then extracted with solvent and analyzed via gas chromatography (GC). Volatiles emitted from control, non-inoculated PDA plates, were also collected and analyzed.

B) One-month-old peanut plants were inoculated with white mold fungus by distributing 5 fungal culture plugs along the main stem of the plant. Agar plugs were made using a # 1 cork borer to cut out portions of fungal culture agar plate. Plants were then covered with 1-gallon plastic ziploc bag and left to incubate for 3 days. Control plants were also covered with the plastic bags for the same period of time, but were not inoculated with the fungus. Headspace air samples were taken from individual whole-plant volatile collection chambers containing either diseased or healthy plants. Volatile emissions were collected for an 18-h interval, and then samples were extracted with solvent and analyzed via GC.

Results: A) The volatile compound 3-octanone was identified from collections made from fungal cultures (Fig 1A). This compound was recovered only from fungal culture plates and not from non-inoculated control PDA plates.

B) Peanut plants infected with white mold fungus emitted a number of volatiles, many of which are released by plants in response to damage by larvae of lepidopteran species (Fig 1B). However, it was noted that the amount of nonatriene released by infected plants is much higher than that previously observed from insect damaged plants. Additionally, diseased peanuts also produced methyl salycilate and this compound has not been recovered, to this date, from plants damaged by lepidopterous caterpillars in studies conducted in our laboratory. The fungal derived compound 3-octanone, though in small quantities, was also recovered from the fungi-infected plants. Non-inoculated control plants released linalool and nonatriene but in significantly lower amounts than those released by white mold-infected plants.

The fact that 3-octanone was recovered from both, fungal culture and diseased plant samples is encouraging because this compound could potentially be key to determine the presence of the pathogen by use of biological or mechanical devices. Additionally, the combination of 3-octanone and methyl salycilate could be used in the future as an indicator of infection in plants and this could facilitate treatment or elimination of affected plants in the field before the disease has a chance to spread and cause significant damage in the field.

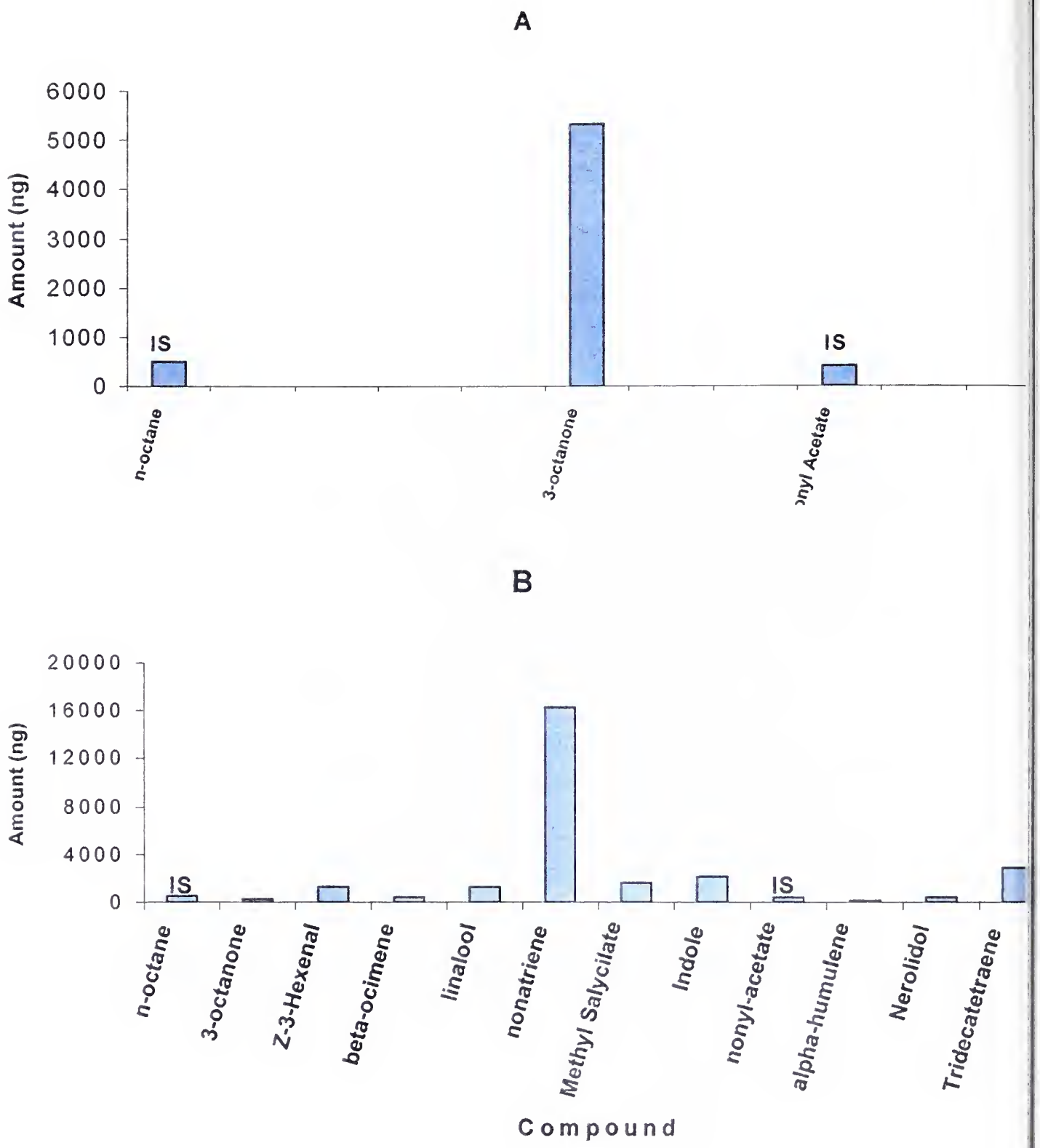


Figure 1- A)Volatiles collected from white mold fungal cultures during a 2 h period. B) Volatiles emitted by peanut plants during an 18 h period, 3 d after inoculation. IS= Internal standard components added to the sample to facilitate quantification of compounds.

CRIS 6615-22000-012-00D

DE-NOVO SYNTHESIS OF INDUCED VOLATILE EMISSIONS IN TOBACCO PLANTS

C.M. De Moraes and J.H. Tumlinson

Objective: The goal of these studies is to understand more clearly how tobacco plants, in response to herbivore damage, activate and regulate over time the synthesis and release of volatile compounds. We also intend to establish whether the release of particular volatiles conforms to specific patterns that may be correlated with parasitoid behavior.

Methods: Eight week old tobacco plants (*Nicotiana tabacun* strain K326) grown from seeds in 16-cm diameter pots were used in all studies. Third-instar tobacco budworm caterpillars (*Heliothis virescens)*, reared on artificial pinto bean diet, were starved for 7 hours, then placed on plants at the start of the experiment and allowed to feed continuously while the plants were in the volatile collection apparatus (see later). Mechanically damaged plants were scraped with a razor blade on the underside of the leaf perpendicular to the vein. The total leaf area and the damaged portions were measured by scanning a photocopy of the leaves (Sigma Scan version 3.0, Jandel Scientific; Sausalito CA.).

In vivo ^{13}C labeling was accomplished by introducing synthetic premixed air containing 360 ppm carbon dioxide (^{13}C 99%), 20.7% oxygen and a balance of nitrogen into the volatile collection apparatus (see later) containing the plant.

Volatile compounds were collected from aerial portions of plants contained within 34-cm tall by 14-cm diameter glass cylinders. Air entered the top of the glass sleeve and passed over the plant at a rate of 1 l min^{-1}. Plant volatiles were collected at 2 hour intervals by pulling half of the air (0.5 l min^{-1}) that had passed over the plant through Super Q adsorbent traps located around the base of the collection chamber; the remainder of the air was vented out the bottom of the system. Temperature and humidity were recorded at 2 minute intervals. Compounds were eluted from the adsorbent traps with 150 µl of CH_2Cl_2; 400 ng each of n-octane and nonyl acetate added as internal standards and 1 µl aliquots analyzed by capillary GC. The amount of ^{13}C incorporated into each compound was determined by chemical ionization (isobutane) GC-MS.

Results: Labeling whole tobacco plants damaged by insect herbivores with $^{13}CO_2$, in tandem with GC-CIMS analysis of volatile compounds released by these plants, provided an account of the overall incorporation as well as a detailed picture of the distribution of the label for each compound. Several compounds incorporated substantial levels of ^{13}C within 5 hours of exposure to $^{13}CO_2$ and were clearly synthesized *de novo* in response to insect damage. These included: (*E*)-b-ocimene (95% ^{13}C), (*E*)-b-caryophyllene (90% ^{13}C), Indole (84% ^{13}C) and a-humulene (90% ^{13}C). During the same 5-hour exposure to $^{13}CO_2$ some of volatile terpenes incorporated very low levels ^{13}C . These included: a-pinene (5% ^{13}C), (*Z*)-3-hexenyl acetate (0%^{13}C). These volatiles were either released from storage, and thus not synthesized in response to insect wounding, or synthesized from other precursor pools, and thus did not incorporate the labeled carbon. The terpene (*E,E*)-a-farnesene (57% ^{13}C), contained intermediate levels of ^{13}C label after 5 hours of $^{13}CO_2$ exposure. Green leaf volatiles of the lipoxygenase pathway, breakdown products of stored lipids, as expected, did not incorporate ^{13}C.

CRIS 6615-22000-012-00D

SYNTHESIS OF VOLICITIN TYPE COMPOUNDS

M. Sammons and J.H. Tumlinson

Objective: To synthesize compounds analogous in structure to the known inducer of plant volatiles, Volicitin (*N*-[17-hydroxylinolenoyl]-L-glutamine) for use in the study of herbivore biosynthesis and plant metabolism of these compounds.

Methods: Various Volicitin type compounds were prepared by coupling the t-butyl esters of amino acids to fatty acids using the method developed by H. Alborn et al.(2000). The protecting t-butyl groups are then removed by acid hydrolysis. Only slight modifications of this method have been necessary to correct for the differing solubility of the conjugates. The method was also scaled down to accommodate 100 mg of ^{13}C-labeled Linolenic acid as starting material in the synthesis of a labeled Glutamine-Linolenic acid conjugate. Purity of the conjugates was determined by HPLC.

Results: Linolenic acid conjugates of Phenylalanine, Asparagine, Valine, Tyrosine, Alanine, Glutamine, and Glutamic acid have been prepared. Linoleic acid conjugates of Phenylalanine, Glutamine, and Glutamic acid have also been synthesized. The Glutamine conjugates of ^{13}C-labeled Linolenic acid and Palmitic have also been prepared.

Each of the conjugates has been synthesized with greater than 95% purity except the Linolenic and Linoleic acid conjugates of Glutamine and Glutamic acid, which have been prepared with purity ranging from 80% to 90%.

Future Plans: The Linolenic and Linoleic acid conjugates of the remaining amino acids will be prepared, as well as cleaner conjugates of Glutamine and Glutamic acid.

Conjugates of Jasmonic acid will also be prepared for use in the study of plant metabolism of volatile inducing compounds.

Alborn, H.T., Jones, T. H., Stenhagen, G. S., and Tumlinson, J. H. 2000. Identification and Synthesis of Volicitin and Related Components from Beet Armyworm Oral Secretions. Journal of Chemical Ecology, 26(1):203-220.

CRIS 6615-22000-012-00D

CHEMICAL ATTRACTANTS OF THE SMALL HIVE BEETLE (*AETHINA TUMIDA*, MURRAY; COLEOPTERA: NITIDULIDAE)

A. Suazo[1] and J.H. Tumlinson

Objectives: A) To find the odor sources that attract small hive beetles (SHB) to a honeybee colony. B) To find and characterize the chemicals in the odor sources that attract the beetles, and C) to find a possible sex or aggregation pheromone that can be used to enhance the efficacy of a potential trap developed using host-produced volatiles.

Methods: A bioassay system using a four arm olfactometer was setup and calibrated to test the attraction of male and female SHB to host and conspecific-produced volatiles. Host produced volatiles evaluated included honey, fresh pollen, bee wax, bee brood and adult worker bees with and without the queen. Male and Female beetles (32 to 64) were tested to these odor sources and their response was evaluated as percentage of time spent per odor zone. Bioassays were also conducted to evaluate the production of a sex or aggregation pheromone by using male and female beetles as odor sources.

Results: Results of the attraction of male and female SHB to host-produced, and conspecific-produced volatiles are shown in Table 1 and 2 respectively. Both male and female SHB are primarily attracted to volatile compounds produced by adult honeybee workers and fresh pollen. Significant differences between male and female attraction to both fresh pollen and adult bees with a queen were observed, however, no differences between males and females attraction was seen when only worker bees were used as odor source. No attraction to any female-produced volatile was seen, however, male-produced volatiles attracted females suggesting the possibility of a sex pheromone produced by males.

Volatiles have been collected from adult worker bees and fresh pollen and are being used to identify the chemicals produced by using thermal desorption coupled with gas chromatography-mass spectroscopic analysis (GC-MS).

[1]Foreign Agricultural Service

Table 1. Response of male and female small hive beetles to host-produced volatiles.

Component. (Odor source)	Sex		Pooled
	Males	Females	
Entire hive	76.3**	59.6**	
Workers and queen	80.1**	48.8**	
Workers only	99.8**	96.8**	
Wax	23.2	32.1	
Brood			26.0
Honey	21.3		
Fresh pollen	47.7**	67.9**	

** : Possible statistical significance
Values are expressed as relative time spent in odor zone source (as percentage) in a four arms olfactometer.

Table 2. Response of small hive beetles to volatiles produced by conspecifics.

Odor source	Sex	
	Male	Female
Males	26.6	48.8**
Females	27.6	31.2

**: Possible statistical significance.
Values are expressed as relative time spent in odor zone source (as percentage) in a four arms olfactometer.

CRIS 6615-22000-012-00D

IDENTIFICATION OF PHEROMONE COMPONENTS IN ORAL SECRETIONS AND CROP OF CARIBBEAN FRUIT FLIES

P.E.A. Teal, F. Lu, and B. Dueben.

Objectives: To determine the role of oral secretions in chemical communication of Caribbean fruit flies

Methods: Oral secretions were collected from male and female Caribbean fruit flies of different ages and extracted with hexane containing internal standard. The hexane extracts were analyzed by gas chromatography and GC-mass spectroscopy to determine the identity and amounts of pheromone components present. Bioassays were conducted by applying 10ul of oral secretions from males on a loquat tree and either 10ul of female oral secretions or just water on another leaf and suspending the leaves in a plexiglass cage. Ten females were introduced into the cage and the behaviors monitored over a 1h period. The crop was excised from males, placed into a conical vial and punctured using a syringe needle prior to addition of hexane containing internal standard and extraction as above. In some cases the liquid was removed by syringe and transferred to another vial. In these cases the crop tissue and crop liquid were extracted and analyzed separately. To determine if suspensolide was converted to anastrephin and epianastrephin in the crop liquid, suspensolide (in acetone/HOH 1:100) or HOH was incubated with crop liquid for 1h. Crop tissue was incubated in 10µl of water for 1 h. Half of the solution was removed and analyzed, while the other half was incubated for one more hour with addition of suspensolide. All samples were extracted and analyzed as described.

Results: Chemical analysis of extracts of the oral secretions from male Caribbean fruit flies resulted in identification of pheromone components including: anastrephin, epianastrephin, suspensolide, bisabolene, and farnesene in a ratio of (63:396:4:8:1). Extracts of the crop from male flies contained these same components. No pheromone was detected in the extracts of female oral secretions. Bioassay of the oral secretions indicated that females were attracted to oral secretions from males but not from females. The amounts of anastrephin and epianastrephin in male oral secretions changed with age and time of the day, and were correlated with the amounts of volatile pheromone components released by male flies. The amounts of suspensolide, bisabolene, and farnesene in the crop tissue were greater than those in the crop liquid, while amounts of anastrephin and epianastrephin in the crop tissue and crop liquid changed during the day. Generally, the amounts of suspensolide and bisabolene decreased, and the amounts of anastrephin and epianastrephin increased from 9 am to 6 PM in both crop tissue and crop liquid. The amounts of anastrephin and epianastrephin from crop tissue or crop liquid incubated with suspensolide were significantly higher than those of control. The data show that oral secretions deposited on leaves by males contain terpenoid pheromone components which attract females and that suspensolide was converted to anastrephin and epianastrephin by enzymatic degradation in the crop of male flies.

CRIS 6615-22000-012-00D

DEVELOPMENT OF AMPHIPHYLIC PSEUDOPEPTIDE ANALOGS OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE

P.E.A. Teal, and R.J. Nachman[1].

Objectives: To design and develop synthetic analogues of insect neuropeptides that penetrate the insect cuticle and maintain bioactivity.

Methods: Pseudopeptide analogs of the C-terminal active core (FSPRLamide) of pheromone biosynthesis activating neuropeptide (PBAN) were synthesized by addition of aliphatic fatty acids including acetic, pentanoic, hexanoic, octanoic, decanoic, dodecanoic and hexadecanoic acids to the aminoterminal phenylalanine. Temporal response studies of pheromonotropic activity were conducted using topical application bioassays in which 500pmol of analogs were applied to the descaled abdomens of females of *Heliothis virescens*. Females were incubated for various times after application. Then the sex pheromone glands were excised, extracted in hexane containing internal standards and the extracts analyzed by capillary gas chromatography to determine the amount of pheromone present. Topical penetration studies were conducted by application of 500pmol of the analogs to excised pieces of cuticle held in wells of microtiter plates previously blocked with gelatin. Cuticle was removed from the plates at various time intervals after application of analogs and internal standard (1nmol PBAN) was added to each well. The contents of the wells were analyzed to determine the amount of analog present in each well using reversed phase microbore liquid chromatography.

Results: The pseudopeptide analogs formed by attachment of acetic, pentanoic or dodecanoic acids induced pheromone production for only 4h after application. Hexanoic, octanoic, and decanoic were capable of stimulating pheromone production for periods of 12h after application. However, an analog formed by attachment of palmitic acid failed to stimulate production of pheromone after as long as 24h. Cuticle penetration studies indicated that both the acetic and pentanoic acid analogs penetrated the cuticle very rapidly which resulted in their ability to stimulate pheromone production in as little as 15min. However, by 6h the amounts of either analog penetrating the cuticle below the lower critical dose for pheromonotropic activity. The C6, C8 and C10 analogs penetrated the cuticle at a relatively constant rate for the initial 8h after application but rates declined significantly between 8-12h after application. Amounts of these analogs penetrating the cuticle after 12 h were below the critical threshold for pheromonotropic activity. The dodecanoic analog penetrated the cuticle relatively slowly but amounts equivalent to the EC_{50} value of 6pmol were found after 1h. Amounts greater than the EC_{95} (12pmol) were present in samples collected between 2-4hr but less than 5pmol were found in samples collected at 6-12h. No detectable amounts of the palmitic acid analog penetrated the cuticle at any interval.

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CRIS 6615-22000-012-00D

EFFECTS OF ALLATOTROPIN AND ALLATOSTATIN ON *IN VITRO* PRODUCTION OF JUVENILE HORMONE BY CORPORA ALLATA OF ADULT FEMALES OF *HELIOTHIS VIRESCENS*

P.E.A. Teal, and A.T. Proveaux

Objectives: To determine the effects of allatotropin and allatostatin on biosynthesis of juvenile hormone homologs by adult females of *Heliothis virescens.*

Methods: Isolated retrocerebral complexes from adult females of *H. virescens* were incubated in tissue culture medium199 containing 2% Ficoll 400, 72 mg/ml $CaCl_2$ and 0.6mM sodium acetate and sodium propionate in a conical amber vial for 12h. Reactions were stopped by addition of methanol and hexane, containing internal standard, and vortexing for 1min. The aqueous and organic layers were separated by centrifugation and the organic layer analyzed by capillary GC-ion trap mass spectroscopy (chemical ionization, isobutane reagent gas) to identify different JH homologs and their amounts. Retrocerebral complexes from newly eclosed females were incubated in culture medium containing 10nmol of either allatotropin or allatostatin or both neuropeptides for 12h prior to extraction and analysis of extracts for the presence and amount of JH produced.

Results: The juvenile hormone homologs JH I, II and III were identified from extracts of tissue incubations of glands from females from the day of emergence until they were eight-days old. Ratios of JH I, II and III did not vary with age but amounts increased in virgins over the first three days then declined. JH III was present in greatest amounts followed by JH II and the amount of JH I present was very low in all samples. Incubation of retrocerebral complexes from newly eclosed females with synthetic *Manduca sexta* allatotropin stimulated significant increases in production of all three homologs but significantly more JHI and JHII were produced relative to JH III. Incubation of glands with allatostatin inhibited production of all three JH homologs. Co-incubation with both neuropeptides resulted in production of significantly less of each of the homologs than when allatotropin was used alone.

CRIS 6615-22000-012-10R

INSECT ELICITOR INDUCED PLANT VOLATILE PRODUCTION: ENDOGENOUS SIGNALS AND PATHWAYS

E.A. Schmelz and J.H. Tumlinson

Objective: To identify plant signal transduction pathways and endogenous regulators which are triggered following plant perception of the insect elicitor volicitin (*N*-(17-hydroxylinolenoyl)-L-glutamine). Isolation and identification of plant signals, collectively called jasmonates, and related conjugates implicated in volatile production will require an optimized bioassay.

Methods: *Plant growth:* Seeds of *Zea mays* L. cv. Delprim were germinated in potting soil for 6 days and transferred to hydroponic containers. All plants were maintained in a 12-h photoperiod with 350 mmol $^{-2}$ s^{-1} of PAR, 70% relative humidity and temperature cycle of 21 °C/ 26 °C (night/day).

Experimental design: We compare (n=6) two bioassay designs (intact and excised), four leaf treatments, three treatment times, and three volatile collection periods. The four leaf treatments consisted of undamaged controls, damage, damage plus volicitin (5 nmole) and damage plus jasmonic acid (JA; 30 nmoles). For the damage protocol, each of the three expanded leaves received two superficial damage sites using a razor to scratch the abaxial surface of the leaves. A total of 18 ml of 50 mM NaH_2PO_4 (pH=8.0) buffer was distributed evenly between the six damage sites on each plant immediately after wounding. Within 20 minutes of the damage treatments, all leaves from the excised groups were cut at the base of the petiole and placed into 4 ml vials of H_2O.

Time of treatment and volatile collection periods. All leaf treatments were performed either 20 minutes prior to the dark cycle, in the middle of the dark cycle or at the initiation of the following light cycle, these periods are designated as 'evening', 'midnight' and 'morning', respectively. All volatiles were collected in three 4 hr periods during the following light cycle. Plants ages differed between the time of treatment, however all plants were 10 days old during the volatile collection.

Volatile collection. Intact and excised plants were placed in tapered glass chambers and assayed under their original lighting conditions. Collection and GC analysis of volatiles followed Turlings et al. 1993.

Results: Over all treatment times, induced volatile production from excised leaves far exceeded those from intact plants. Volatile production in undamaged leaves did not differ between excised and intact bioassays, however damage and elicitors stimulated volatile production in excised leaves between 1.5 to 8-fold higher than identical treatments in intact plants (Figure 1). Leaves treated and excised at 'midnight' produced the greatest quantity of volatiles during the following 12 hr light cycle. Time of treatment greatly influenced both the quantity and ratio of volatiles produced. Independent of the bioassay type or time of treatment, volicitin induced volatile production is far more transient than JA induced volatile production. Based on this data, future studies will examine volicitin induced changes in endogenous jasmonates from excised bioassays treated in the middle of the dark cycle.

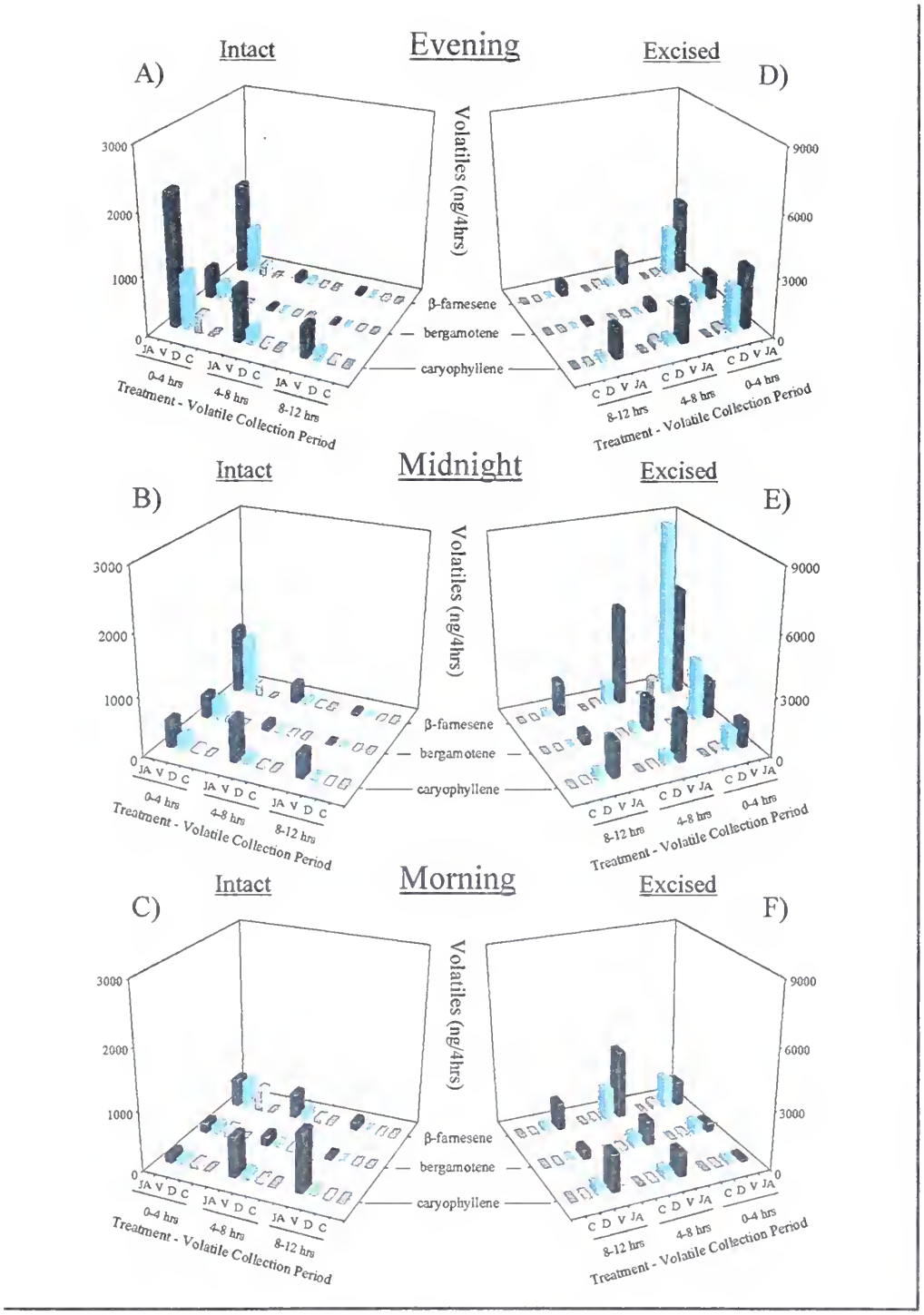

Figure 1. Average (n=6) quantity (ng/4 hrs) of b-farnesene, bergamotene, and caryophyllene released from intact (A-C) and excised (D-F) corn seedling bioassays. Leaves were either undamaged (C) or damaged and treated with buffer containing water (D), volicitin (V; 5 nmoles/plant) or jasmonic acid (JA; 30 nmoles/plant). Evening, Midnight, Morning indicate time of leaf treatment while volatiles were collected during the following photoperiod in three 4hr time periods (denoted 0-4, 4-8, 8-12hrs).

CRIS 6615-22000-012-12S

[1]CHARACTERIZATION OF DAMAGE-INDUCED VOLATILES FROM SOLANACEOUS PLANTS IN THE FIELD

A.M. Redman[1] and J.H. Tumlinson

Objective: This research is intended to provide evidence that plants release damage-induced volatiles under field conditions and that volatiles are correlated with the attraction of parasitoids and elevated parasitism of herbivores. Our research will also include wild, native plants.

Methods: *Plants.* Preliminary research has utilized eastern black nightshade (*Solanum ptycanthum*), as well as tomato (*Lycopersicon esculentum*, cv. "Mountain Delight"). Experiments with native plants take place both in wild populations and, along with tomato, in fields where seedlings have been transplanted. All plants from the latter group are started in potting soil from seed in the greenhouse and then transferred to the field at the four-leaf stage. Once planted, all plants are watered daily for the first week and during periods of drought thereafter, and tomato plants are fertilized monthly. Plants in natural populations are left undisturbed (except for periodic removal of plants immediately neighboring our experimental plants, which is necessary to ensure access with sampling equipment).

Herbivore damage. Plants were damaged with *Manduca sexta* (Lepidoptera: Sphingidae) second and third instars, beginning at the start of the experiment and continuing during the sampling period; caterpillars were replaced before they molted to the fourth stadium, and control plants were left undamaged. In future experiments, *M. sexta* larvae will be placed on individual treatment plants the evening before volatile collection for those plants commences; further, plants will be transplanted in experimental field plots at intervals, allowing us to sample all field season from plants of the same age. In natural populations, existing plants can be grouped into age classes for a similar approach.

Volatile collections. Plants are enclosed in sample bags, and volatiles are pulled across Super Q-filled traps at a rate of 20% of the air circulated through the bags (1L/min out, and 5L/min filtered air in) for three-hour intervals, three times per day, from 9am to 6pm. Current research is underway to compare results obtained from sample bags constructed of Teflon with those constructed of nylon. We have only four pumps at this time, limiting us to two treatment-pairs per day (two treated plants and two controls).

Parasitoid attraction. Air not pulled out through filters is blown out of sample bags onto yellow sticky traps through Teflon-lined Tygon tubing, and parasitoids caught on these traps are counted. Also, plants are damaged by *M. sexta* but not subjected to volatile sampling, and sentinel *M. sexta* larvae, glued to popsicle sticks during the molt from second to third instar are hung from the plant canopy where they are available for foraging parasitoids. Larvae are allowed to molt and then reared for one week before freezing, dissection, and counting parasitoids.

Results: This project is still under development, and results are preliminary. However, we do have evidence that *C. congregata* is attracted to volatiles associated with damaged nightshade and tomato plants in the field: the number of wasps caught on sticky traps blown with air from sample bags covering damaged plants (*mean* = 12.2 and 8.1 for tomato and nightshade respectively) were significantly higher ($p < 0.05$ in χ^2 contingency analysis) than numbers caught on traps receiving air from control plants (mean = 4.5 and 3.2 for tomato and nightshade respectively).

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IMPORTED FIRE ANT

AND

HOUSEHOLD INSECTS

CRIS - 6615-32000-033-00D–Reduced-Risk Integrated Management of Medically-Important Household Arthropod Pests

CRIS - 6615-32000-034-00D-Risk Assessment and Integrated Termite Management Strategies for Hawaii and the Pacific Basin

CRIS - 6615-32000-035-00D-Biologically-Based Integrated Strategies for Management of the Imported Fire Ant

CRIS 6615-32000-033-00D

PROCEDURES FOR ESTIMATING SPATIAL / TEMPORAL RISKS USING POLYCLONAL ELISA ASSAYS FOR COCKROACH ANTIGENS AND COCKROACH TRAP COUNTS

R.J. Brenner, D.A. Focks, S. Lele[1], E. Horowitz[2], V. Rice[2], M. Anderson[3], and A. Togias[2]

Objectives: In the indoor environment, cockroach proteins are among the two highest triggers / inducers of asthma in the U.S. Our research goal is to have environmental assays for cockroaches and cockroach antigens suitable for assessing spatial / temporal risks to occupants. To accomplish this, assays must be sensitive, and be analyzed in a manner that allows us to adequately evaluate spatial variability of antigens in discrete areas.

Methods: In 5 urban Baltimore homes, sticky traps were emplaced (1 per 1.5 m^2) to measure comparative infestation rates for subsequent correlation to spatial distribution of antigen load. Antigens were sampled at locations by swabbing 10 x 10 cm areas on a 45 cm grid spacing in selected rooms. Antigens were measured using a polyclonal rabbit-anti-cockroach ELISA inhibition assay with standard curves derived from extracts of German cockroach debris from USDA colonies. A second standardization curve utilized swabs from petri dishes containing timed exposures of known numbers of mixed stages of German cockroaches to yield cockroach-hour equivalents. Each assay is evaluated using 4 replicates in 2 microtiter plates.

Results: Three homes showed some degree of infestation; a comparison of average antigen values from 490 swab samples to average trap catches from 72 traps indicated a general proportional trend. In the most heavily infested home (mean trap catch of 3.45 cockroaches / trap) number of cockroaches by room (Y) were regressed from average antigen values in swabs by room (X); the regression equation was highly significant ($Y=0.237+0.79(X)$; $r^2=.998$). Within rooms, however, there were obvious areas where cockroach population distributions did not match antigen loads, based on a spatial dynamics index comparing probability contours describing spatial distributions of approx. 80% of antigen loads vs. 80% of cockroach distribution. These estimates were done at resolutions of 10 cm^2, and illustrate the need for a sensitive assay to measure antigens in 2-dimensional space, independent of monitoring current cockroach populations. These data now will be used to estimate the optimal number of samples and the optimal sampling locations that will yield satisfactory prediction information on the entire antigen load in 2-dimensional space. Resultant calculations will be used – within the context of time / space budgets of occupants – in risk assessment analysis to identify areas of high antigen load (spatial hot spots, displayed as areas of high risk) that can be targeted for subsequent intervention efforts. Work will continue in Baltimore under an ARS / Johns Hopkins funded by NIH, and in Cleveland under a Cooperative Agreement with ARS / Environmental Health Watch, funded by HUD.

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CRIS 6615-32000-033-00D (also 034-00D)

IMPROVING MONITORING AND BAITING TECHNOLOGIES FOR NATIVE AND INTRODUCED SUBTERRANEAN TERMITES

D.A. Focks, D.W. Woodson[1], R.J. Brenner, C.S. Oman, and K. McRae

Objective: The primary focus of near-term research is to improve the detection of subterranean termite activity and the introduction of toxicants into colonies in residential areas. A recent and promising development in the control of subterranean termites involves baiting with a slow-acting toxicant. Effectiveness is a function of many variables but the acceptability and the spatial location of baits are seen as key factors warranting additional research. Currently, only about 10% of baits placed around the perimeter of a structure are ever found by foraging workers. This low rate significantly limits the amount of toxicant that can be introduced into a colony and hampers control. The field studies being conducted in Gainesville are designed to provide insight into foraging behavior and baiting strategies with the goal of increasing the frequency with which baits are found. A modest increase in bait finding from 10% to 15 or 20% would increase toxicant delivery by 50 to 100%. This work is being done in collaboration with the Southern Region Research Center, New Orleans, LA allowing us to address this important area for native and Formosan subterranean termites.

Anecdote and some indications in the literature suggest that foraging activity is not random but is more likely to occur in areas of conducive conditions- the presence of moisture, certain types of ground cover and food. Soil moisture is a hydrologic balance involving gains (irrigation and rainfall), retention (soil type and ground cover), and loss (aspect of building and shading vegetation and structure). It may be possible to significantly improve the rate at which monitoring devices are found by selectively locating them on the basis of these conducive conditions. We term variables that are either significantly positively or negatively correlated with activity as co-variates.

Methods: Field experiments involve placing wooden stakes every 1 to 10 meters around the perimeters of buildings and monitoring them monthly for termite activity for a period of 12 to 24 months. At each stake location, the value for each potential co-variate is recorded. Spatial and traditional (logistic regression) statistical analysis is used to develop equations relating the probability of a stake being found and the significant co-variates; the end use will be to develop a set of easy-to-use guidelines to aid pest control technicians in the placement of baits. If these studies indicate that easily-observed co-variates can improve stake placement, we will consider the development of spatially-based software to run on small palm-sized computers that would automate and facilitate bait location; several national pest control companies have expressed strong interest in this approach. Study sites include 13 building on the UF campus and approximately 250 houses currently being monitored by Orkin. Two higher resolution studies are underway involving the area-wide gridding (4-m spacing) in a ca. 1 ha. campus site and a 1-m resolution study of 6 residential sites in Gainesville.

Results: To date, we have records on approximately 1,000 stakes around UF campus buildings and their associated co-variates. Preliminary analysis suggests that it will be possible to develop easy-to-use guidelines for pest control industry technicians that will improve the rate at which monitoring and baiting devices are found.

[1]USDA-ARS, Southern Regional Research Center, New Orleans, Formosan Termite Project

CRIS 6615-32000-033-00D

DEVELOPMENT OF BAITS FOR THE CONTROL URBAN PEST ANTS

D.H. Oi and D.F. Williams

Objective: Ants in and around buildings has become a major problem of homeowners and managers of commercial buildings. Ant control is currently the number one priority for the pest control industry. Using baits is the most efficient method of ant control. Due to the diversity of food preferences and habitats, a single bait is not effective for all pest ant species. Thus, there is a tremendous interest in developing new ant baits for pervasive urban pest ants such as the Argentine ant, carpenter ant, white-footed ant and Pharaoh ant. In cooperation with manufacturers, several ant attractants and active ingredients have been examined for attractiveness and efficacy in an effort to develop new ant baits.

Methods: Candidate food-based bait attractants formulated as liquids, granules, or gels were exposed to several pest ant species to assess their attractiveness. All formulations did not contain active ingredients. Tests were conducted on laboratory colonies of the following ants species: Argentine ants (*Linepithema humile*), ghost ants (*Tapinoma melanocephalum*), big-headed ant (*Pheidole megacephala*), Crazy ants (*Paratrechina longicornis*), red imported fire ants (*Solenopsis invicta*), Pharaoh ants (*Monomorium pharaonis*), and the Florida carpenter ant, *Camponotus floridanus*. Bait attractancy was determined in choice tests by determining the number of ants on the attractants at 10, 20, 30, 60, 90, and 120 minutes and at 24 hrs. Baits were weighed before and after the 24 hour exposure to estimate consumption. Sets of baits were held, without exposure to ants, for the 24-hour period to estimate weight changes due to evaporation or moisture absorption.

To evaluate the efficacy of several liquid, granular and gel formulations containing various combinations of attractants and concentrations of active ingredients, standard laboratory bioassays were conducted using ant colonies of Pharaoh ants, red imported fire ants, or Argentine ants. Test materials were generally exposed for 3 days to colonies before a standard diet was added. Efficacy was determined from weekly assessments of adult ants, brood (immature ants), and queen survivorship for 4 to 8 weeks.

Results: Liquid baits were fed upon more heavily than the solid and gel bait formulations for the majority of the species. Feeding on the gel bait was statistically comparable to the liquids for the lipid feeding ant species (red imported, Pharaoh, and big-headed ants). Two proprietary formulations of a granular ant bait resulted in greater than 80% reductions in adults and 90% reduction in brood. Queen death was also observed. These formulations are promising candidate baits that eventually may be marketed to the pest control industry.

CRIS 6615-32000-033-00D (also 034-00D)

INSECTICIDE TOLERANCE IN THE FORMOSAN AND DARK SOUTHERN SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

W.L. A. OSBRINK[1], A.R. LAX[1], and R.J. BRENNER

Objectives: Determine lethal time to mortality responses for seven insecticides against workers and soldiers of the Formosan subterranean termite, Coptotermes formosanus Shiraki, and workers of the dark southern subterranean termite, Reticulitermes virginicus (Banks).

Methods: Four colonies of C. formosanus and four colonies of R. virginicus were obtained from field sites in New Orleans, LA and north FL, respectively. Termites were tested for their sensitivity to insecticides by exposing them to a Whatman filter paper surface treated with known amount of toxicant dissolved in 50.0 ml of spectrophotometric grade acetone, and the acetone was allowed to evaporate. Toxicants used were chlordane, methoxychlor, chlorpyrifos, permethrin, cypermethrin, deltamethrin, bendiocarb, and fipronil. Ten third instar or greater workers, as determined by size, or soldiers were placed in four similarly prepared vials (replicates). Mortality was recorded, until at least 90% were dead, at intervals of 5 min and at longer intervals if the tests progressed beyond 100 min. Data from the four replicates were pooled and analyzed by probit analysis for lethal time to 50% mortality (LT50's) and 90% mortality (LT90's) Tolerance ratios (TR) of two estimated LT90's doses and their 95% confidence limits for i = 1,2 were calculated based on estimates for the intercepts (a) and slopes (B) of two probit lines and estimates of their variance-covariance matrixes as follows: Ratio = 10a; lower limit = 10a-2s; upper limit = 10a+2s;with qi = (1.28-ai)/Bi; and var (qi) = (1/Bi2)[var (ai) + 2qi cov (ai, Bi) + qi2 var (Bi)]; with a = q1 - q2 and s = [var (q1) + var (q2)]1/2 (Robertson and Preisler 1992). This TR estimation procedure adjusts for lack of parallelism of LT lines. A tolerance ratio was considered significant if the 95% confidence limits excluded one.

Results: There were significant differences in the tolerance ratio between workers of C. *formosanus* colonies to all toxicants tested except fipronil. One colony was 16X more tolerant than another to deltamethrin. *C. formosanus* soldiers had significant differences in tolerance ratios among colonies exposed to all toxicants except chlorpyrifos. Chlordane, methoxychlor, permethrin, and fipronil did not kill soldiers from some colonies. Some *R. virginicus* colonies were significantly more tolerant than others to chlordane, methoxychlor, chlorpyrifos, permethrin, cypermethrin, bendiocarb, and fipronil, but not deltamethrin. In some *C. formosanus* colonies the worker LT curves displayed substantial flattening in response to permethrin, and deltamethrin. Lethal time curves for *C. formosanus* soldiers exposed to chlordane, chlorpyrifos, permethrin, cypermethrin, deltamethrin, and bendiocarb showed substantial flattening. *R. virginicus* workers demonstrated substantial flattening when exposed to chlordane, methoxychlor, chlorpyrifos, deltamethrin, and fipronil. These findings indicate that substantial inter-colony tolerance to insecticides exists.

[1]USDA-ARS, Southern Regional Research Center, New Orleans, Formosan Termite Project

CRIS 6615-32000-033-00D

MEMBRANE-BOUND ESTERASES RESPONSIBLE FOR CYPERMETHRIN RESISTANCE IN THE GERMAN COCKROACH

S.M. Valles

Objective: In a recent survey of field-collected German cockroaches, DEF pretreatment reduced the cypermethrin resistance level in 83% of populations tested. In addition, general esterase activity was well correlated (r=0.78) with cypermethrin resistance magnitude. These data implicated widespread esterase involvement in cypermethrin resistance in the German cockroach. Hence, cypermethrin metabolism studies were conducted using a pyrethroid-resistant strain of German cockroach to gain a more complete understanding of the role esterases in resistance.

Methods: The German cockroach strain used in this study was collected by vacuum from a single family home in Gainesville, Florida. The strain was maintained in 8 liter glass jars with rolled cardboard for use as harborage, water, and #5001 laboratory rodent diet. Orlando, the standard insecticide-susceptible German cockroach strain, was used for comparison with the Aves strain in all assays.

Cypermethrin metabolism studies were conducted with the 105,000 g_{Max} supernatant (soluble fraction) and pellet (microsomes) prepared from adult males of the Orlando (S) and Aves (R) German cockroach strains. The 2 ml reaction mixture contained 50 m*M* sodium phosphate buffer, pH 7; 0.5 mg of protein; and 12,000 dpm (0.04 µg) of [^{14}C]cypermethrin in 40 µl of ethylene glycol monomethyl ether. For oxidative metabolism, the reaction mixture was fortified with an NADPH-generating system; esterase activity was inhibited by adding DEF (10^{-4} M) to the reaction mixture. Duplicate incubations were carried out at 28° C in a shaking water bath for 30 minutes. Cypermethrin and its metabolites were extracted with diethyl ether, separated by thin layer chromatography and quantified by liquid scintillation spectrometry.

Results: Synergist data had previously implicated enhanced hydrolytic metabolism as a resistance mechanism in the Aves German cockroach strain. This conclusion was further supported by significantly greater cypermethrin detoxification by microsomal esterases from the Aves strain. Therefore, enhanced cypermethrin metabolism catalyzed by microsomal esterases can be considered a major mechanism of cypermethrin resistance in the Aves strain. This is the first report of microsomal esterase involvement in insecticide resistance in the German cockroach. Preliminary purification of these esterases has indicated a qualitative difference in isozyme expression between the cypermethrin-resistant and -susceptible strains.

CRIS 6615-32000-033-00D (also 034-00D)

TOXICITY AND *IN VITRO* METABOLISM OF *TRANS*-PERMETHRIN IN THE EASTERN SUBTERRANEAN TERMITE

S.M. Valles and R.J. Brenner

Objective: Despite near complete reliance on insecticides for subterranean termite control, little is known about their ability to detoxify these chemicals. Several species possess competent oxidative, conjugative, and hydrolytic detoxification systems. However, direct measurement of insecticide metabolism as it compares with insecticide toxicity is lacking. Moreover, little information is available concerning comparative insecticide susceptibility among different colonies of the same subterranean termite species. Therefore, the toxicity of *t*-permethrin and *in vitro t*-permethrin metabolism studies were conducted in *Reticulitermes flavipes* to gain a better understanding of the detoxification ability of this economically important species.

Methods: Insecticide bioassays were conducted by placing termites on a residue of *t*-permethrin. Qualitative and quantitative *in vitro t*-permethrin metabolism experiments were conducted with the 105,000 g_{Max} supernatant (soluble fraction) and pellet (microsomes) derived from termite workers. Three enzyme sources were used: (1) microsomes plus an NADPH source and DEF (microsomal monooxygenase activity), (2) microsomes (microsomal esterase activity), and soluble fraction (cytosolic esterase activity).

Results: The UF *R. flavipes* colony was 2-fold more tolerant of *t*-permethrin than the ARS colony at the LC_{50}. *t*-Permethrin was metabolized *in vitro* by microsomal monooxygenases, microsomal esterases, and cytosolic esterases prepared from worker termites. Metabolism by microsomes in the presence of an NADPH source and DEF to inhibit esterases (i.e., microsomal monooxygenases) was qualitatively identical for the UF and ARS colonies; both produced 3 metabolites with R*f* values of 0.75, 0.60, and 0.47. No metabolism was observed when NADPH was excluded from the reaction mixture. Quantitative metabolism by microsomal monooxygenases, as measured by total metabolite production, was not significantly different (t = 0.67, df = 4) for UF and ARS colonies. Similar results were observed when cytosol was used as the enzyme source. No significant differences in the rate of *t*-permethrin metabolism or in the metabolites produced were observed. Finally, quantitative and qualitative microsomal esterase metabolism was similar between strains. Both colonies produced two major metabolites (Rf 0.15, 0.13) and metabolized *t*-permethrin at a rate of 33.0 ±3.7 and 36.3 ±6.2 pmol/h/0.5 mg protein, respectively. Metabolism by microsomes (without NADPH) and cytosol was prevented by the addition of DEF to the reaction mixtures.

CRIS 6615-32000-035-00D

DEALATION IN *SOLENOPSIS INVICTA* FEMALE ALATES

S.N. Burns, R.K. Vander Meer, and P.E.A. Teal

Objective: Investigate and define the developmental parameters that influence dealation in fire ant female alates, pre- and post-mating.

Methods: Newly-eclosed (sexually immature) and 7- and 14-day-old *S. invicta* alates (sexually mature) were placed in queenless monogyne colony units, each consisting of equal amounts of worker adults and brood. Alates were observed over 12-h periods for indications of wing casting, defined as the removal of at least three of four wings. Sexuals were removed upon dealation to eliminate the release of pheromone responsible for inhibiting dealation of cohabiting alates. In subsequent experiments, sexually mature alates were isolated individually and in groups in test tubes half-filled with moistened cotton. Alates were observed in the same manner described above.

The rates of dealation were observed for alates displaying a variety of behaviors associated with a mating flight. Four categories of alate behavior were examined: 1) excited alates scurrying on the surface of the soil; 2) excited alates climbing onto tongue depressors; 3) tethered alates flying for 5min under laboratory conditions, but not primed for a mating flight; and 4) alates displaying all of the identified pre-mating behaviors. Alates displaying similar behaviors were placed in groups. Alates were observed for dealation according to procedures described above.

Since dealation is thought to be related to increased Juvenile Hormone (JH) levels in the hemolymph we compared the size of the corpora allata (CA, the source of JH) of newly-eclosed and 14-day-old alates, and uninseminated 19-day-old dealates. Alate and dealate brains were excised from the head cuticle to expose the CA and placed on microscope slides. The area of an individual corpus allatum was measured using an ocular micrometer.

Results: Sexual maturity was not a major factor influencing the time in which alates shed their wings. However, the exclusion of workers and brood did have an effect on the rates of dealation, in that alates in isolation and those in groups shed their wings later than alates in the presence of workers and brood. Workers were not observed physically assisting alates in wing casting, but tactile and olfactory stimuli or food may be potential factors provided by workers and brood to stimulate dealation. None of the examined pre-mating behaviors were found to stimulate dealation comparable to that of newly-mated queens, suggesting that mating, alone or in combination with other behavioral and/or environmental cues, may be a major factor inducing the rapid post-mating dealation.

The sizes of CA did not change once alates reached sexual maturity. In addition, there were no differences between the sizes of CA of alates and those of dealates. These results suggest that no correlation exists between CA sizes and the production and/or release of JH into the hemolymph in *S. invicta*.

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NEW HOST FOR THE PARASITIC ANT SOLENOPSIS DAGUERREI (HYMENOPTERA: FORMICIDAE) IN ARGENTINA

L.A. Calcaterra, J.A. Briano, and D.F. Williams

Objectives: The ant, *Solenopsis daguerrei*, is a parasite of fire ants in South America. It lacks a worker caste, so all adults are reproductive males and females. The parasitic queens attach themselves to the host queens, and divert resources from them. The fire ant workers tend *S. daguerrei* in a manner similar to their own mother queens which can inhibit the egg production of the fire ant mother queens causing the fire ant colony to collapse and eventually die. Thus, this parasite is a candidate for introduction for the biological control of imported fire ants in the U.S. However, before *S. daguerrei* can be released in the U.S., a major objective was to determine its host specificity.

Methods: A field host range survey was conducted in San Eladio (60 km W of Buenos Aires), Argentina, the only place where *S. daguerrei* has been found consistently since 1995. This area had the highest abundance (7 % of fire ant colonies) of *S. daguerrei* recorded in South America to date. The surveys were conducted from December 1996 to May 1997 and from November 1997 to May 1998 and consisted in walking through the pastures to visually detect the fire ant colonies. When a colony was found, it was excavated, scattered on the ground, and throughly examined for *S. daguerrei* adults. Some colonies were excavated and put into 10-liter buckets for separation in the laboratory by flotation, and then placed in rearing trays for later examination. Alcohol samples were kept of most species found. Samples of parasitized fire ant colonies were preserved in hexane to confirm their identification by gas chromatography analysis of cuticular hydrocarbons and venom alkaloids.

Results: Previous surveys on natural enemies of fire ants in South America revealed that *S. daguerrei* has been found in Argentina, Uruguay, and Brazil in colonies of *S. richteri*, *S. invicta*, *S. saevissima*, and *S. macdonaghi.* In this study, a total of 4,316 ant colonies of 9 different species in 4 subfamilies were sampled. Of these, 96 % were fire ants. *S. daguerrei* was found exclusively in 161 colonies of fire ants. Taxonomic studies revealed that 95 % of the parasitized colonies corresponded to the fire ant, *S. richteri,* however, the remaining 5 % was identified as *Solenopsis quinquecuspis.* This is the first report of *S. quinquecuspis* as a host of *S. daguerrei.*

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SEASONAL STUDIES OF AN ISOLATED RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE) POPULATION IN EASTERN TENNESSEE

A.A. Callcott, D.H. Oi, H.L. Collins, D.F. Williams, and T.C. Lockley

Objectives: Studies of an isolated 1200 hectare infestation of imported fire ants in eastern Tennessee were initiated in an effort to learn more about its adaptability in a more northern climate. Winter survivability and impact on local ant fauna were evaluated over a 4 year period.

Methods: Winter kill (survival) of red imported fire ant (RIFA), *Solenopsis invicta*, colonies in Tennessee site was compared with a control site in Mississippi using the USDA Fire Ant Population Index method (Lofgren and Williams, 1982, J. Econ. Ent. 75: 798-803) on eight 0.1 hectare test plots per site. For the impact studies of the RIFA on the local ant fauna, four transects, 200 meters long, were placed in the fire ant infested areas and three were placed in non-infested areas. Each transect transversed similar habitats and efforts were made to include as much habitat diversity as possible. These transects were used for both bait and pitfall traps. Bait and pitfall traps were alternated along each transect at 10 meter intervals. Evaluations were conducted once in 1993, three times in 1994 and 1995, twice in 1996, and once in 1997. Weather data for the Tennessee site was supplied by the property owner, Bowater Corporation and also by a National Weather Bureau station 16 kilometers away while weather data for the Mississippi site was supplied by the National Weather Bureau station at Saucier, MS.

Results: In the initial sampling (1993), both the Tennessee and the Mississippi (control) sites had similar population indices and number of RIFA colonies present. During the sampling periods in 1994, a severe winter kill of RIFA colonies occurred in the Tennessee site and the populations did not rebound over the following summer months. Populations finally begin to rebound the following summer (1995) but never reached the initial numbers when the final evaluation were made in 1997. We found that in severe winters, the largest colonies survived the best and that winter survival of RIFA may be more dependent on the overwinter mean maximum temperatures rather than the mean minimum temperatures in an area. In other words, successful overwintering of RIFA was most influenced by the degree of sustained, near freezing weather. Conversely, the RIFA populations in the Mississippi site were adversely affected by weather in the summer months when high temperatures were sustained for 2 - 3 months. A comparison of other ant species present in RIFA infested and non-infested sites in Tennessee found that RIFA had less impact on ant species density and diversity than other studies. This was probably due to the low population of RIFA following the severe winter kill during the first winter of our study.

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CONSIDERATIONS FOR PLANNING, IMPLEMENTING AND EVALUATING A SPOT-ERADICATION PROGRAM FOR IMPORTED FIRE ANTS

B.M Drees, H. Collins, D.F. Williams, and A. Bhatkar

Objectives: The imported fire ant infest much of the southeastern United States and parts of some western states (New Mexico, Arizona, and California). In large infested areas, eradication is not currently feasible because current treatment methods require all infested areas to be treated. Any untreated infested areas within miles of these treated areas will serve as a source of re-invasion. To date, there has been no documented case of a successful imported fire ant eradication program, although numerous isolated, spot infestations have been treated. Some newly-infested areas which are disjunct or isolated from large infested areas could be prime candidates for attempting spot-eradication efforts to prevent or remove the area from federal quarantine. This document was developed to present considerations for spot-eradication in newly-infested, isolated areas.

Methods: Previous studies, documented cases, and literature reviews were conducted to obtain the most recent information of attempts at fire ant eradication including spot eradications. We reviewed publications and references of past large scale eradication programs related to the imported fire ant.

Results: Imported fire ant suppression in large acreage is currently only feasible using chemical methods that use broadcast applications of bait-formulated insecticides. When properly applied, these products eliminate about 90% of the fire ant mounds within a period of weeks to months, and their effects can last up to a year, depending on the product selected, re-invasion potential due to climatic conditions and the size of the treated area. As a result, multiple broadcast applications of the bait product(s) would be required in order to begin to approach 100% "control". Only in small isolated areas of infestation (e.g., less than a few acres) should the use of individual ant mound treatments be considered as a treatment approach. Otherwise, mound treatments should only be used as a part of a "two-step method" which relies on the periodic broadcast applications of ant bait products. Most bait products are not registered for use in all sites in which fire ants occur, and none can be applied directly to bodies of water or wetlands. A theoretical treatment for a spot-eradication program should include the following elements:

—Areas considered for an attempted spot-eradication should not have other areas of infestation within 2 to 5 miles (e.g., a reasonable distance beyond the length of most mating flights - although with prevailing winds extending flights, this distance should be increased) which could serve as a source for re-invasion.

—ALL infested lands within the area will require treatment.

—Multiple broadcast applications of one or more bait products will be required for several years in any reasonable attempt to achieve 100 percent control.

Once treatments have been initiated, periodic monitoring of populations will be necessary to document the success of treatments by establishing and maintaining permanent sampling plots where ant mounds can be assessed in areas of known size (e.g., 1/4 acre circular plots). Upon completion of the treatment regime (two to three years), sampling efforts should be continued for an additional two years before determining that the spot-eradication program was a success.

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CHANGES IN THE CUTICULAR HYDROCARBON PROFILE OF THE SLAVE-MAKER ANT QUEEN, *POLYERGUS BREVICEPS*, AFTER KILLING A *FORMICA* QUEEN

C.A. Johnson[1], R.K. Vander Meer, and B. Lavine[2]

Objective: To determine the mechanism by which newly mated queens of the obligate slave maker ant, *P. breviceps*, successfully gets through host colony defenses, finds and kills the host colony queen, and then is accepted by host colony workers.

Methods: Whole ant colonies and newly mated queens of the parasitic ant, *P. breviceps*, and its two host *Formica* species (*F. gnava* and *F. occulta*) were collected from the Chiricahua Mountains of southeastern Arizona and set up in the laboratory using standard procedures. Encounters between parasite and host were induced and the parasite queen and the usurped host queens were sacrificed for gas chromatograph (GC) analysis of cuticular hydrocarbons. Specimens were soaked in hexane for 10 minutes and the extract was concentrated and applied to a Pasteur pipet silica gel column, that was then eluted with hexane to isolate the cuticular hydrocarbons. The complex mixture was separated into its components by GC. This provided quantitative data. Further analysis using GC/mass spectroscopy was used to identify the individual components. Comparison of the groupings (parasite, hosts, and each of the latter after usurpation by the parasite) was accomplished using multivariate principal component analysis of the GC data.

Results: Queens of the slave-maker ant, *Polyergus breviceps*, take over nests of their *Formica* host species by fatally attacking the resident queen. As workers only begin grooming the *P. breviceps* queen once she has ceased her attack, we investigated whether a transfer of chemicals from host queen to parasite queen may be responsible for this dramatic change in worker behavior. The cuticular hydrocarbons were found to be a mixture of normal, methyl, and dimethyl branched compounds, ranging in length from C_{24} to C_{29}. We determined that the cuticular hydrocarbon pattern for newly mated *P. breviceps* queens and for queens of their two *Formica* host species were species-specific and found that the cuticular hydrocarbon pattern of the parasite queen changed markedly after attacking a host queen. The newly acquired profile was virtually identical to the queen profile of the species killed. Principal component analysis of the cuticular hydrocarbon profiles of parasite and hosts visually indicates the hydrocarbon pattern change that takes place in newly mated *P. breviceps* queens after attacking a *Formica* host queen. These results suggest that cuticular compounds from the hosts are transferred to the parasite queen during their aggressive interaction and that this transfer is correlated with successful host colony usurpation by the parasitic queen.

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EXPANDING HABITAT OF THE IMPORTED FIRE ANT (SOLENOPSIS INVICTA): A PUBLIC HEALTH CONCERN

S.F. Kemp, R.D. deShazo, J.E. Moffitt, D.F. Williams, and W.A. Buhner II

Objectives: The increase in densities and continued spread of the imported fire ant has led to a rise in attacks on animals and humans. Recent reports of fire ants invading buildings such as health care facilities and stinging occupants has heighten public health concerns. This report reviews the medical entomology, clinical aspects of stings, and the current approaches to chemical control of the imported fire ant..

Methods: Entomological references, clinical records, case series and literature reviews were conducted to obtain the most recent information of indoor fire ant attacks on humans. We reviewed medical entomological references related to imported fire ants, clinical records, legal depositions, newspaper reports and other material related to fire ant stings, habitat expansion, and present control techniques.

Results: The ultimate range of the fire ant is unknown but based on recent estimates, they will inhabit at least 25% of the continental United States. The USDA estimates that imported fires ant have expanded westward approximately 120 miles per year. Because they are highly mobile, establish colonies in diverse habitats, and are difficult to detect when they invade new areas, it may be 3-4 years before a new infestation is detected. Because they favor disturbed habitats, the progressive urbanization of the U.S., especially in the Sun Belt, has accelerated their expansion. Imported fire ants seek nesting sites for colony survival during environmental stress, such as food shortages, hot and dry summer periods or heavy rainfall. Inhabited dwellings can be ideal locations because they can provide food, moisture, and protection from weather extremes. As fire ants move indoors, they may come in contact with humans and sting the residents. Typically, 30% to 60% of subjects in infested urban areas are stung each year by imported fire ants. However, one survey reported stings in 89% of subjects or immediate family members per year. Furthermore, 51% previously unexposed subjects were stung within 3 weeks of arrival in an endemic area, and16% developed fire ant venom-specific IgE antibody. Stings occur most frequently during the summer, most commonly in children, and typically on the lower extremities. Imported fire ant venom differs from bee, hornet, and wasp venoms, which are largely protein-containing aqueous solutions. Ninety-five percent of fire ant venom is water-insoluble alkaloid, the remaining aqueous fraction contains soluble proteins that comprise only 0.1% of the venom by weight. Chemicals are currently the only effective measures. Basic methods are broadcast applications, usually using a bait, individual mound treatments, or both. In addition, chemical barriers and spot treatments may be helpful in some situations. Imported fire ant stings in health care facilities or public buiidings attract legal attention. Physicians in such cases are often asked to render an opinion concerning the quality and appropriateness of patient care. For these reasons, physicians in endemic areas should familiarize themselves with the risk management issues related to the medical consequences of imported fire ant stings.

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IMPORTED FIRE ANT CONTROL DEMONSTRATION FOR TROPICAL FISH FARMS

D.H. Oi and D.F. Williams

Objective: To dDevelop a program for controlling imported fire ants in tropical fish farms. The production of tropical (ornamental) fish for aquariums accounts for over half of the value of Florida aquaculture sales. In Florida there are over 200 active growers of tropical fish, and at most farms, fire ants are not controlled because of the fear of detrimentally impacting fish being raised in ponds. Fish farmers typically work barefoot along the pond banks and because the banks provide an ideal habitat for fire ant colonies, the probability of being stung is very high.

Methods: The banks of fish ponds at the University of Florida Tropical Aquaculture Laboratory in Ruskin, FL, were surveyed for red imported fire ant mounds. The area around 23 ponds was treated with a fire ant bait that contained the insect growth regulator methoprene. This bait (Extinguish) was selected because of its low toxicity to fish and that methoprene formulations are used for mosquito control in aquatic ecosystems. In March 2000, bait was broadcast between ponds with a Herd Seeder attached to an all terrain vehicle typically used at these facilities. Two areas encompassing 8 ponds were used as untreated controls.

Results: Percent reductions of active fire ant mounds in the treated area from the initial survey made in March 2000 were 60 and 63% at 15 and 22 weeks post-treatment. In contrast, the untreated control areas had an 83 and 63% INCREASE in the number of active fire ant mounds. Post-treatment mound counts in the treated area was 25 and 23 mounds. In the untreated controls mounds numbered 55 and 49 for the two post-treatment surveys. Employees at the facility perceived a substantial reduction in the amount of fire ants. Subsequent surveys will be made to determine when fire ants reinfest the area and another bait application is required. While bait particles were observed to land in some ponds, no detrimental affects on fish were noted.

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PARASITOID-HOST MATCHING BETWEEN THE LITTLE DECAPITATING FLY *PSEUDACTEON CURVATUS* FROM LAS FLORES, ARGENTINA AND THE BLACK FIRE ANT *SOLENOPSIS RICHTERI*

S.D. Porter and J.A. Briano

Objective: Matching biotypes of potential biocontrol agents to target host populations can greatly improve the effectiveness of control. This study was designed to determine if the fly *Pseudacteon curvatus* Borgmeier from Las Flores, Buenos Aires Province, Argentina prefers its natural host, the black fire ant, *Solenopsis richteri* Forel. This information will help us determine whether to focus release efforts with this fly against black, red, or hybrid populations of imported fire ants.

Methods: Flies used in this study were originally collected from El Toro Ranch southeast of Las Flores, Buenos Aires Province, Argentina. To examine *P. curvatus* preferences for *S. richteri*, *S. invicta,* and hybrid fire ants, flies were introduced into white plastic trays with screened vents and tight-fitting glass lids. In the bottom of each tray, were two parallel chambers for two species of ants. We used 7 colonies of *S. richteri* from Las Flores, Buenos Aires Province, Argentina, 9 colonies of *S. richteri* from northeastern Mississippi, and 7 colonies of hybrid fire ants from around Starkville, MS. For each trial *S. richteri* and hybrid fire ants were paired with similar-sized *S. invicta* workers from Gainesville, FL. Different colonies were used for each trial to assure that results were not due to differences in the attractiveness of individual colonies. Each test run lasted about 3 h and used 14-18 female flies with an equivalent number of males. Test ants contained 0.25 g of workers (~400) and 0.5 g of brood. The trays were inspected every 10 min and the number of female flies hovering in attack mode over each species of ant was recorded by visual count.

To determine if *P. curvatus* flies were equally successful in parasitizing black, hybrid, and red fire ants, we conducted a series of no-choice parasitism tests. The trays used in these tests contained a single solid bottom covered with moistened plaster. We conducted 6 trials each. Each trial lasted 2 days. At the end of each trial, test ants were placed in a small box. and dying workers were inspected every 1-2 days for fly pupae for a period of 30 days.

Results: We found that *P. curvatus* strongly preferred black fire ants from Argentina, imported black fire ants from the United States, and hybrid imported fire ants from the United States when each was tested against red imported fire ants from the United States (Fig. 1). Parasitism rates, however, were not significantly different among these ant hosts in no-choice parasitism tests. These results suggest that while the decapitating fly, *P. curvatus*, may do well against all kinds of imported fire ants, it may do best against black or hybrid fire ant populations.

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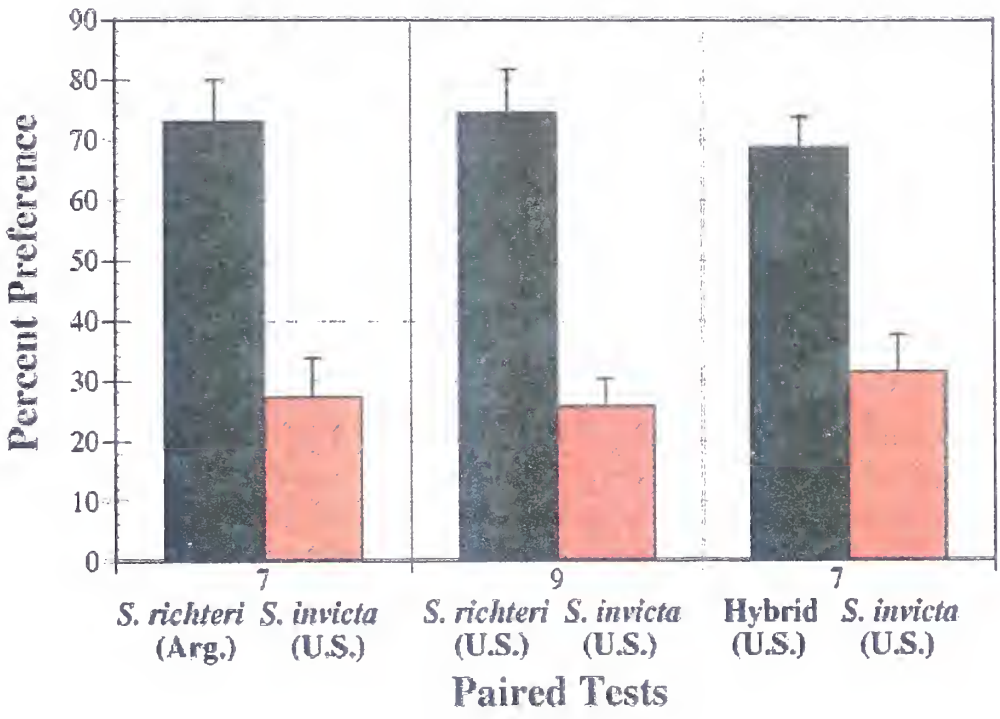
Fig. 1 The percent of female *Pseudacteon curvatus* decapitating flies from Las Flores Argentina preferring to attack *S. richteri* from Argentina, *S. richteri* from Mississippi, or hybrid (*S. richteri* x *S. invicta*) fire ants from Mississippi, each in paired tests with *S. invicta* from Gainesville, Florida. The number of trials is indicated below each pair of bars. Error bars indicate standard errors of the mean.



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FIELD RELEASES OF FIRE ANT DECAPITATING FLIES IN THE SOUTHEASTERN UNITED STATES: PROGRESS REPORT FOR 1999-2000

S.D. Porter, L.A. Nogueira de Sá, H.L. Collins, K. Flanders, C.S. Gorsuch, F. Graham, S.J. Johnson, K. Kidd, J. Kintz, T.C. Lockley, R.M. Pereira, and J.T. Vogt

Objective: *Pseudacteon* flies are common parasitoids of fire ants in South America where fire ant populations are generally very low compared to the United States. Our field releases of the decapitating flies, *Pseudacteon tricuspis* and most recently *Pseudacteon curvatus,* are designed to help determine where these flies will survive, how rapidly they expand out of their release sites, and what impacts they are having on imported fire ant populations in the United States.

Methods: In 1999, we released *Pseudacteon tricuspis* at 8 new sites in 6 states: Florida (2), South Carolina (2), Alabama (1), Louisiana (1), Tennessee (1), and Texas (1). In 2000, we released *P. tricuspis* at 11 sites in 8 states: Alabama (1), Florida (2), Georgia (1), Louisiana (2), Mississippi (1), North Carolina (1), Oklahoma (2), and South Carolina (1). In addition we also released a second *Pseudacteon* species for the first time, *P. curvatus*. This very small species was released at 8 sites in Florida (4), Alabama (1), and Tennessee (3). At each site, we released 2,500-5,000 flies over a 10-12 day period. *P. tricuspis* flies were released in groups of 40-60 over fire ant workers in large plastic trays (2 by 4 feet). *P. curvatus* flies were allowed to parasitize fire ant workers in the lab for several days before they were returned to the field.

Results: All five of the 1999 sites with flies in the fall survived the winter and still have flies. This was especially significant because it showed that this species is capable of surviving winters in central Alabama (Auburn) and even more importantly far western South Carolina (Clemson). Of the three failures, one was probably due to only releasing a small number of flies (1,200 flies) Myrtle Beach, SC); the other two (TX, TN) may be related to drought conditions or incompatibility with local ants. Flies at the Auburn, AL site have begun to expand out of their release site and are now 2-5 miles in all directions.

Five of the 2000 *P. tricuspis* releases have multiplied in the field and survived through to the fall (FL, SC, LA, MS, NC). Releases at two sites may have failed (FL, OK). Releases at the other sites were made within the last month so we will not know how they are doing until late fall or next spring. Releases of *P. curvatus* appear to have been less successful. All four Florida sites appear to have failed as well as two sites in Tennessee. One site in Tennessee is still in progress. However, *P. curvatus* flies released at the Alabama site (Talladega) have survived the summer and at least 3-4 generations in the field. This is the first release of *Pseudacteon* flies that appears to be surviving on hybrid fire ants (*S. invicta* x *S. richteri*).

Our 1997-1998 releases in Florida are firmly established and expanding at the rate of 3-4 miles per year. Last fall we estimated that they covered almost 50 square miles; this fall we estimate that it will be over 100 square miles. To monitor the impacts of phorid flies on fire ant populations in Florida we set up 36 treatment sites this spring along the expanding wave front and another 36 control sites 20 miles to the north in similar habitat.

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ILLUSTRATED KEY TO *PSEUDACTEON* DECAPITATING FLIES (DIPTERA: PHORIDAE) THAT ATTACK *SOLENOPSIS SAEVISSIMA* COMPLEX FIRE ANTS IN SOUTH AMERICA

S.D. Porter and M.A. Pesquero

Objective: Scientists have described 18 species of *Pseudacteon* decapitating flies in South America that attack fire ants in the same species complex as those accidently imported into the United States almost 70 years ago. In order to facilitate identification and study of this important group of biocontrol agents, we published an illustrated key to all 18 species. Previous keys were incomplete, poorly illustrated and difficult to use.

Results: The genus *Pseudacteon* was described by Coquillett (1907). Female *Pseudacteon* flies are characterized by having fully developed wings, large eyes with hundreds of ommatidia, more than 4 bristles on the frons, unforked wing veins, hind tibia with a dorsal hair palisade, and a symmetrical highly sclerotized ovipositor.

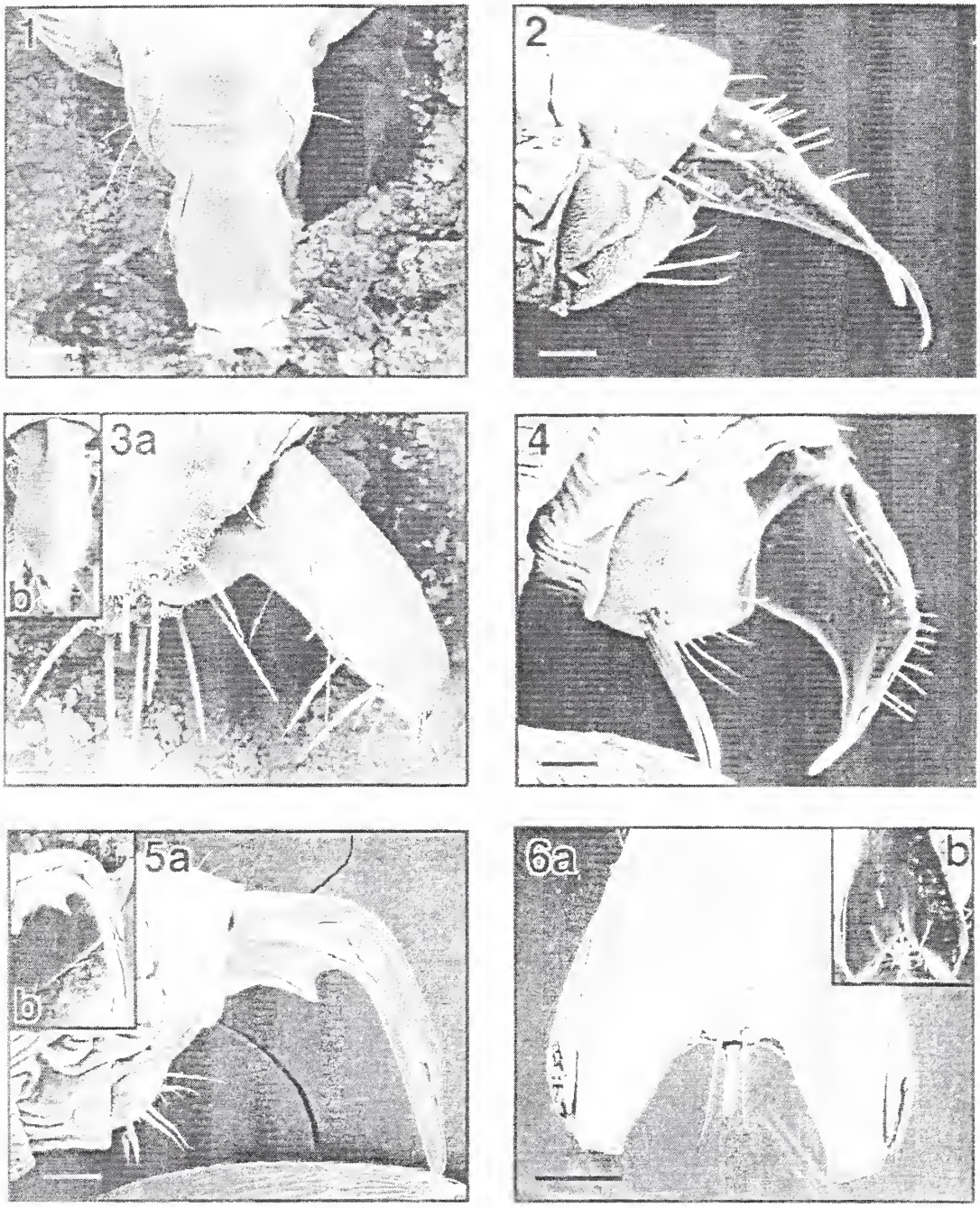
This paper provided an illustrated key to all described species of *Pseudacteon* flies that are known to attack *Solenopsis saevissima* complex fire ants. Female decapitating flies are most easily identified by their highly distinctive ovipositors (see Fig. 1-6).

Phorid flies in the genus *Pseudacteon* are of particular interest because of: 1) their potential use as classical biocontrol agents for imported fire ants in the United States and other parts of the world and 2) their unusual life history. *Pseudacteon* flies appear to be promising biocontrol agents because fire ants utilize a suite of highly specific defenses against attacking flies. These defenses could only have evolved and be maintained if *Pseudacteon* flies were having evolutionary impacts on fire ant populations or sexual production.

Pseudacteon flies are the kind of parasitoid that gives science fiction writers fodder for their stories. Adult females dive in and inject their torpedo-shaped eggs into the bodies of ant workers. The developing maggot moves into the head of its host where it develops for 2-3 weeks. Just prior to pupation, the host worker is decapitated. The maggot consumes everything in the head and pupates inside the empty head capsule, using it as a pupal case.

Several species illustrated in our key exhibit regional variability (e.g., Fig. 5-6). Some cases may be intraspecific clinal variation while other cases may be true sibling species isolated by geography or host preferences. The significance of regional variation is largely unknown; however, different biotypes of the same species or sibling species can be specialized to attack different fire ant hosts. Matching appropriate *Pseudacteon* biotypes to imported fire ants in the United States is likely to be important in their success as biocontrol agents.

Figs. 1-6. Electron micrographs of the ovipositors of 6 of the 18 species of *Pseudacteon* decapitating flies that attack close relatives of imported fire ants in South America. **Fig. 1**. Dorsal posterior view of *P. conicornis.* **Fig. 2.** Lateral view of *P. solenopsidis.* **Fig. 3.** *P. convexicauda*; a) lateral view; b) dorsal posterior view. **Fig. 4.** *P. borgmeieri,* lateral view. **Fig. 5.** *P. curvatus,* lateral views. **Fig. 6.** Dorsal-posterior views of *P. nudicornis.*



CRIS 6615-32000-035-00D

IMPROVING MASS REARING OF THE DECAPITATING FLY *PSEUDACTEON TRICUSPIS*

S.D. Porter, R.A. Smith, D.A. Nordlund, and R.K. Vander Meer

Objective: Rearing decapitating flies is a labor-intensive process. Improving our abilities to rear flies allows us to release more flies at more sites.

Methods: As part of our ongoing efforts to improve the efficiency of our rearing procedures, we 1) constructed large automated attack boxes that reduced setup and handling time and 2) developed procedures to eliminate hand collection of fly pupae. Three large automated attack boxes were constructed to replace the 25 small attack boxes. Each box was 3 ft by 8 ft by 18 inches high. The boxes were designed to hold 14 trays of ants. In order to keep the ants active and exposed to fly attacks, we constructed a pneumatic motor and pulley system that automatically raised a refuge cup in one end of each tray while at the same time lowering a refuge cup in the other end of the tray. A timer was set to repeat this cycle every 12 minutes. This apparatus kept the ants running back and forth from one end of each tray to the other throughout the day. Hand collecting fly pupae with forceps and a miniature vacuum system was the most labor-intensive part of the rearing procedure. In order to eliminate these labor costs, we developed a system of plating pupating larvae on trays (10 by 40 cm) and storing them in humidified racks until they were ready to emerge. The trays were then transferred out to the large attack boxes described above.

To improve fly production efficiency, we 1) investigated the use of brief non-lethal electric shocks to release chemicals that stimulate flies to attack (See report by Vander Meer and Porter for details), and 2) looked at the effects of providing new host ants three times a week rather than twice a week. We were able to provide new host ants 3 times a week because of the greatly reduced setup time required for the large attack boxes.

Results: The large automated attack boxes saved us about 3 h/wk in set up time and another 3 h/wk in time spent transferring flies from emergence boxes to attack boxes. This allowed us the time to try providing new host ants three times a week rather than just twice a week The big attack boxes also allowed us to change from running attacks 6 days a week to 7 days a week without additional labor. These boxes were also large enough to permit tests with unsorted pupae and chemical attraction. We found that the trick to avoiding hand sorting the pupae was to keep the dead ants and fly pupae very moist and ignore the buildup of mold on the trays. This new procedure will save us 10-15 hours per week and will make feasible a much larger rearing effort planned in cooperation with USDA-APHIS and the Florida Division of Plant Industries.

As described in detail in a following report, the use of electrical shock to release chemical attractants increased production rates by almost 20%. Providing new host ants three times a week rather than twice a week improved production by about 30% These increases in production rates were especially important because they more than doubled the number of flies available for release after subtracting the number of flies needed to maintain colony production.

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POWERFUL QUEEN INFLUENCE ON CONSPECIFIC FIRE ANT, *SOLENOPSIS INVICTA*, AGGRESSION

R.K. Vander Meer and L.E. Alonso

Objective: To systematically investigate the remarkable decrease in conspecific aggression observed in *Solenopsis invicta* workers when the colony queen is removed.

Methods: Experiment 1: Queenless monogyne and polygyne worker subunits were created from queenright colonies and the change in conspecific worker aggression over time was recorded. Experiment 2: Queenless worker groups from monogyne and polygyne colonies (N=30 for each group) were collected from the field and maintained in the laboratory using standard procedures. Newly mated *S. invicta* queens (NMQs) were collected immediately after mating flights in the vicinity of the USDA laboratory in Gainesville, Florida. The high weight of the NMQs (>14mg) indicated that they were from monogyne colonies. Within three hours of collection, NMQs were introduced into monogyne queenright colonies. The aggressive response of workers to the introduced NMQs was recorded based on a standard aggression scale. Experiment 3: NMQs were introduced into monogyne queenless worker subunits collected directly from the field. The aggressive behavior of resident workers toward the intruder NMQs was recorded. Experiment 4: Under similar conditions to Experiment 3, NMQs were introduced into queenless monogyne and polygyne worker subunits that had previously adopted a NMQ (now queenright; Vander Meer, Alonso, and Anderson, in preparation) and the aggressive behavior of the workers was recorded.

Results: Monogyne fire ant colony workers are territorial and are aggressive toward members of other fire ant colonies. In contrast polygyne colony workers are not aggressive toward non-nestmates, presumably due to broader exposure to heritable and environmentally derived nestmate recognition cues (broad template). In contrast, after fire ant mating flights, newly mated queens are heavily preyed upon by workers from existing monogyne and polygyne fire ant colonies, thus limiting potential reproductive competition. We discovered that existing monogyne and polygyne queens have a remarkable effect on conspecific recognition. After removal of their colony queen, monogyne worker aggression toward non-nestmate conspecifics quickly drops to merely investigative levels; however, heterospecific recognition/aggression remains high. Queenless monogyne or polygyne worker groups were not aggressive toward newly mated queens. Queenless worker groups that adopted a newly mated queen became aggressive again toward non-nestmate workers and newly mated queens. We propose that the powerful effect of fire ant queens on conspecific recognition is caused by a queen produced recognition primer pheromone. This primer pheromone results in the regulation of exogenous reproductive competition in S. *invicta*. The lack of worker/worker aggression in polygyne populations falls within the overall conspecific recognition primer pheromone and is a result of the broad nestmate recognition template associated with polygyne fire ant populations. This extraordinary discovery has broad implications regarding monogyne and polygyne colony and population dynamics.

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ADOPTION OF NEWLY MATED QUEENS BY QUEENLESS MONOGYNE AND POLYGYNE *SOLENOPSIS INVICTA* COLONIES

R.K. Vander Meer, L.E. Alonso, and J. Anderson

Objective: To determine if the drop in conspecific aggression in fire ant workers when they lose their queen enables them to adopt one or more newly mated queens.

Methods: Monogyne and polygyne worker fragments were collected from field sites identified with each social type in and around Gainesville, FL. Each queenless worker fragment was weighed and the number of workers adjusted to approximately 700mg. The colony fragments were maintained in the laboratory by standard methods. Newly mated *S. invicta* queens (NMQs) were collected immediately after mating flights in the vicinity of the USDA laboratory in Gainesville, Florida. The high weight of the NMQs (>14mg) indicated that they were from monogyne colonies. Within three hours of collection, groups of five NMQs were carefully introduced into the following situations. Experiment #1: Five NMQs were added to laboratory reared queenright monogyne and polygyne colonies and their fate recorded. Experiment #2: Five NMQs were added once to queenless *S. invicta* monogyne and polygyne colony fragments, and the fate of the NMQs recorded. Experiment #3: Five NMQs were added on three separate occasions to queenless *S. invicta* monogyne and polygyne colony fragments, and the fate of the NMQs recorded. The date of NMQ addition was dictated by mating flight activity. Experiment #4: NMQs were added to queenless worker groups that had adopted at least one NMQ, and the fate of the NMQs was recorded.

Results: Newly mated queens (NMQs) were executed within three days in every instance by workers when introduced into laboratory maintained queenright monogyne or polygyne colonies. However, in dramatic contrast, workers that were queenless for two days or 15 days prior to NMQ introduction maintained significantly more newly mated queens three days after introduction then queenright colonies, regardless of the worker's social origin – monogyne or polygyne. The percent of NMQs surviving when introduced into monogyne or polygyne worker groups that had previously adopted at least one NMQ was significantly less than those surviving with the same worker groups without a queen. Attrition of the added NMQs proceeds at a steady linear rate for the duration of the experiment. For control groups of one or five NMQs set up without workers (claustrally) there is initially "natural" NMQ attrition until the first workers (nanitic workers) emerge as adults. At this point NMQ attrition for the five NMQs accelerates, presumable due a combination of queen/queen and worker/queen aggression until there is a single queen. The pattern of NMQ survival in formerly queenless monogyne and polygyne worker groups was significantly different from the pattern of NMQ survival when five NMQs were grouped claustrally. The biomass produced by five NMQs founding together was significantly greater than that of single NMQs founding colonies. The biomass produced by NMQs adopted by monogyne or polygyne worker groups was significantly greater than that produced by either of the claustrally founding control groups. The rapid execution of NMQs by monogyne worker groups that have already adopted a NMQ precludes the former from adding to colony biomass in the situations where there were more than one addition of NMQs. Some queenless colonies adopted multiple NMQs and maintained them well beyond the time at which all control groups with 5 NMQs had gone to a single queen, thus we have artificially created polygyne colonies in the laboratory.

CRIS 6615-32000-035-00D

ATTRACTION OF PARASITIC PHORID FLIES TO *SOLENOPSIS INVICTA* SEMIOCHEMICALS

R.K. Vander Meer and S.D. Porter

Objective: To determine if parasitic phorid flies are attracted to semiochemicals released by their fire ant host, *Solenopsis invicta*.

Methods: In the Laboratory: Petrie dishes were fitted with two independent electrical grids composed of 2mm wide copper tape, such that a walking fire ant worker would be able to close the electrical circuit. A standard electrical plug was attached to one end of standard electrical cord and the two wires at the other end were soldered to the ends of the copper tape grid. The apparatus was plugged into a Variac Voltage regulator, which in turn was plugged into a standard 120-volt outlet. Control Petrie dishes were without the electrical grid. Automated rearing chambers contained two rows of seven (7) fire ant colony trays, each with workers and brood. Centrally located trays, 3, 5, 10, and 12 were selected as Treatments (2) and Controls (2). Pre-counts were made of phorid flies attacking and at rest in the treatment and control trays. Fire ant workers (50-100) were placed in the control and treatment Petrie dishes and the current turned on (60-70 volts) for 30 sec, then off for 30 sec for a total of 3 minutes. The number of active flies in the trays and those at rest were counted after a total of 5 and 10 min. In the field: A field site known to have a sustaining phorid fly population was selected for field evaluation of the ability of shocked fire ant workers to attract phorid flies. Treatment Petrie dishes described above were randomly placed ca. 20 meters apart. The treatment electrical outlets were energized using a 15-volt to 120-volt inverter attached to an automobile 15-volt outlet. Control Petrie dishes were placed ca. 3 meters from the treatment dishes. Fire ant workers (100-200) were placed in the control and treatment Petrie dishes as described above. Pre-counts of phorid flies at treatment and control Petrie dishes were made, then treatment Petrie dishes were energized as described above. Phorid fly counts were made 3 and 8 minutes after the start of the experiment.

Results: An electrically energized grid induced fire ants to release exocrine gland products that 1) activated resting phorid fly parasites; 2) guided activated phorid flies to the source of the emitted chemicals; and 3) increased the number of phorid flies attacking fire ant workers. In laboratory rearing chambers with electrically stimulated fire ants, the number of attacking flies was increased by greater than six fold. The number of resting flies was decreased by over 50%. The electrical grid system was adapted to field conditions as described above. Electrical grids with fire ant workers attracted an average of 4 phorid flies/replicate, 3 minutes and 8 minutes after initial energization. No flies were observed at the controls. Thus, volatiles released by worker fire ants activated and attracted phorid flies to the source of the released chemicals. Both the laboratory and the field results point toward practical applications. Phorid flies are labor intensive to rear in the laboratory; therefore, increasing phorid fly rearing efficiency by increasing their attack rate is very desirable. The methods currently used to monitor phorid fly populations in the field are labor intensive. Our discovery, especially in combination with a trapping system, may provide a labor saving method for phorid fly detection and monitoring. Also, the activating / attraction power of the released chemicals can be used to focus existing phorid fly populations into specific areas of interest or aid in the search for phorid fly biotypes in South America that respond best to the semiochemicals released by our imported fire ants.

MOSQUITO

AND

FLY

CRIS - 6615-32000-031-00D--Repellent Systems and Control Strategies for Mosquito/Vectors of Medical and Veterinary Importance

CRIS - 6615-32000-032-00D--Biological Control and Integrated Management of Bloodsucking and Nuisance Flies of Med/Ag/Vet Importance

CRIS 6615-32000-031-00D

FIELD EVALUATION OF THREE DEET-ALTERNATIVES FOR REPELLENCY TO *AEDES TAENIORHYNCHUS* IN THE EVERGLADES NATIONAL PARK USA

D.R. Barnard, U.R. Bernier, K.H. Posey, and R.D. Xue

Objective: This is a report of field tests of the repellency of KBR 3023, IR 3535, Natural Insect Repellent (NIR) (*p*-menthane, 3-8-diol) and deet against black salt marsh mosquitoes (*Aedes taeniorhynchus*) in the Everglades National Park, Florida, USA.

Methods: Deet, KBR 3023, and IR 3535 were tested as 25% solutions in EtOH. NIR was tested as a proprietary formulation (40% PMD). EtOH was used as the control and also to determine mosquito biting rates. Five human subjects were used in 5 tests (three 7 AM-1 PM tests and two 2 PM-8 PM tests). No subject received the same treatment twice. One milliliter of repellent mixture was applied to a randomly selected forearm of each subject and spread evenly to cover 650 cm^2 of skin between the wrist and elbow. Gloves, a headnet, boots and pants, and a long sleeve shirt were worn to prevent mosquito bites on other body areas. Morning and evening tests each comprised 3 minute counts of biting mosquitoes on the forearm of each subject (treated and control) once per hour for 6 hours. Percent repellency was calculated as: (#biting mosquitoes on: (control arm - treated arm)) / #biting mosquitoes on control arm. The complete protection time (CPT) provided by each repellent was also calculated as the time elapsed (in hours) between repellent application and the observation period *immediately* preceding the first mosquito bite. Test data were compiled for statistical analysis so that each record contained a test date, a morning or evening test time, the observation period, the name of the human subject, the treatment received, and the number of mosquito bites received on the forearm (if any). Log transformed data were used to minimize heteroscedasticity in mosquito biting rate responses. Thus, in calculating % R and in the analysis of variance, each biting response datum (n) was transformed to $\log_{10}(n + 1)$ before analysis. Means separation was via Tukey's procedure.

Results: *Biting rates;* (a) The variance in biting rate responses (untransformed data) was related to the mean biting rate; that is, large mean biting rates were accompanied by large variances. This finding justified the use of log-transformed biting rate data in the statistical analysis of percent repellency. (b) The overall mean biting rate (untransformed data) of *Aedes taeniorhynchus* on the untreated (control) subjects during the test was 13.5 per minute. *Percent Repellency:* (a) Deet, KBR 3023, IR 3535, and NIR each provided $\geq$ 65% repellency throughout the test. (b) The ethanol control provided 0% repellency throughout the test. (c) None of the repellents tested, including deet, provided 100% repellency for 6 hours. (d) The overall $\log_{10}$ mean repellency of KBR 3032 was significantly greater than IR 3535 and NIR. (e) The overall $\log_{10}$ mean repellency of deet did not differ significantly from KBR 3023, IR 3535, or NIR. *Complete Protection Time:* (a) The ethanol control provided 0 hours of protection against biting mosquitoes. (b) KBR 3023 provided the longest mean CPT against biting mosquitoes. (c) IR3535 provided the shortest mean CPT against biting mosquitoes. (d) The mean CPT of IR3535 against biting mosquitoes was significantly less than KBR 3023. (e) The mean CPT of deet against biting mosquitoes was not significantly different from KBR 3023 or NIR.

CRIS 6615-32000-031-00D

KETONES ALONE AND IN COMBINATION WITH L-LACTIC ACID AS ATTRACTANTS FOR THE YELLOW FEVER MOSQUITO (*AEDES AEGYPTI*)

U.R. Bernier, D.L. Kline, K.H. Posey, and D.R. Barnard

Objective: The discovery of highly efficient attractant blends for mosquitoes will improve surveillance accuracy for mosquitoes that are vectors for the transmission of various diseases to humans. Additionally, the discovery of blends that do not require carbon dioxide to attract mosquitoes will result in surveillance that is less expensive and more readily deployed in the field. Therefore, the attraction of *Aedes aegypti* to ketones was examined, including combinations of a ketone plus L-lactic acid to identify synergistic binary blends. Many of the ketones tested are known to be present on human skin, and these compounds could possibly function in a similar role to that of carbon dioxide, i.e. as a means to excite and activate mosquitoes.

Methods: A triple cage, dual port olfactometer was used to assess the level of attraction of laboratory-reared, 6 to 8 day old nulliparous female *Ae. aegypti* mosquitoes. Air drawn from outside the laboratory is filtered, cooled or heated, and humidified or dehumidified as necessary by the air handling system to produce a constant airstream through the olfactometer of 80°F and 60% relative humidity. Bioassays were conducted three times per day (8:30, 11:00, 13:00 hrs local time). Mosquitoes were loaded and allowed to acclimate in the olfactometer at least 60 min prior to each of the bioassay times. The mosquitoes trapped in the baited and unbaited ports, and those remaining in the cage were counted after each 3 min bioassay. Data were recorded as a percentage of the mosquitoes attracted to the baited port out of the total number of mosquitoes initially in the cage. Treatments consisted of methanolic L-lactic acid at 200 mg, plated out and dried 3 min on the inner surface of a scintillation vial cap (1.6 cm^2). Ketones were dispensed into a separate smaller vial cap, with an inner surface area of 0.62 cm^2. The ketones were not subjected to a drying period. The blank port for each test contained no treatment but had identical apparatus consisting of caps used to hold the treatments and an identical tray to hold the caps.

Results: Acetone and butanone were the only two ketones that exceeded L-lactic acid in attraction of *Ae. aegypti*. All of the ketones showed at least some ability to attract the mosquitoes; however, the attraction was very weak. When the ketones were combined with L-lactic acid, binary blends with acetone and butanone were synergistic at the dose level use in our experiments. The attraction to blends with 2-pentanone, 3-pentanone, and 3-hexanone was found to be additive (the attraction of the blend was approximately equal to the summed attraction levels of the single components). Combinations of L-lactic acid with 2-nonanone, 2-decanone, or 4-decanone resulted in attraction much lower than expected; these three ketones are weak inhibitors. It was determined from additional experiments that the addition of butanone to a binary blend of L-lactic acid and acetone did not further enhance the binary mixture. Butanone is postulated to perform the same function as acetone in the attraction of mosquitoes, just at a slightly less efficient level. Currently, it is thought that the ketones, such as acetone and butanone, in part perform the same role as carbon dioxide, i.e. to excite mosquitoes.

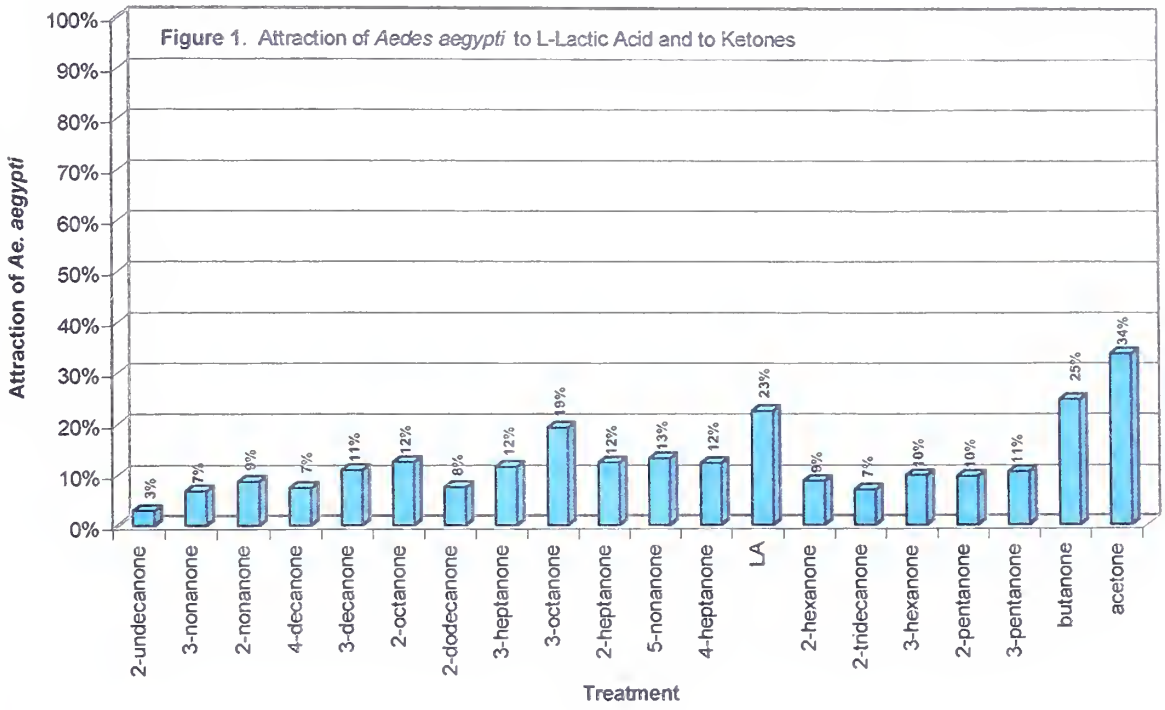
Figure 1. Attraction of Aedes aegypti to L-Lactic Acid and to Ketones
Attraction of Ae. aegypti
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
3%
7%
9%
7%
11%
12%
8%
12%
19%
12%
13%
12%
23%
9%
7%
10%
10%
11%
25%
34%
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3-nonanone
2-nonanone
4-decanone
3-decanone
2-octanone
2-dodecanone
3-heptanone
3-octanone
2-heptanone
5-nonanone
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3-pentanone
butanone
acetone
Treatment

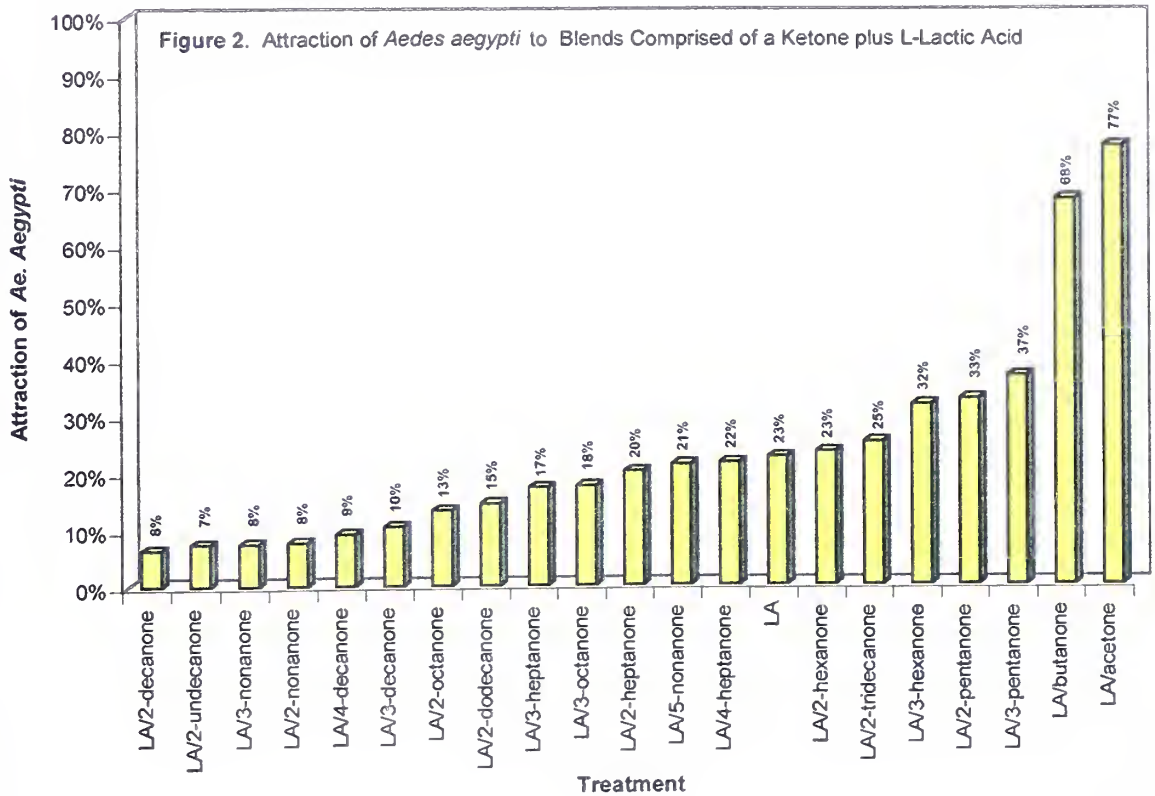


Figure 2. Attraction of Aedes aegypti to Blends Comprised of a Ketone plus L-Lactic Acid
Attraction of Ae. Aegypti
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
8%
7%
8%
8%
9%
10%
13%
15%
17%
18%
20%
21%
22%
23%
23%
25%
32%
33%
37%
68%
77%
LA/2-decanone
LA/2-undecanone
LA/3-nonanone
LA/2-nonanone
LA/4-decanone
LA/3-decanone
LA/2-octanone
LA/2-dodecanone
LA/3-heptanone
LA/3-octanone
LA/2-heptanone
LA/5-nonanone
LA/4-heptanone
LA
LA/2-hexanone
LA/2-tridecanone
LA/3-hexanone
LA/2-pentanone
LA/3-pentanone
LA/butanone
LA/acetone
Treatment

CRIS 6615-32000-031-00D

DISCOVERY OF A COMPOUND ON HUMAN SKIN THAT INHIBITS THE ABILITY OF MOSQUITOES TO DETECT AND LOCATE HOSTS

U.R. Bernier, K.H. Posey, D.L. Kline, and D.R. Barnard

Objective: The discovery of an inhibitor or spatial repellent will provide an additional means of personal protection form mosquitoes responsible for the transmission of various diseases to humans. The discovery that humans produce inhibitors in addition to attractants of mosquitoes results in a better understanding of the complex interaction of chemicals used as odor cues for host-seeking. Therefore, the inhibitor compound was tested for its ability to mask odors produced by humans, and its ability to mask odors from our most effective synthetic attractant blend to demonstrate its ability to perform as a spatial repellent.

Methods: A triple cage, dual port olfactometer was used to assess the level of attraction of laboratory-reared, 6 to 8 day old nulliparous female *Ae. aegypti* mosquitoes. Air drawn from outside the laboratory is filtered, cooled or heated, and humidified or dehumidified as necessary by the air handling system to produce a constant airstream through the olfactometer of 80°F and 60% relative humidity. Bioassays were conducted three times per day (8:30, 11:00, 13:00 hrs local time). Mosquitoes were loaded and allowed to acclimate in the olfactometer at least 60 min prior to each of the bioassay times. The mosquitoes trapped in the baited and unbaited ports, and those remaining in the cage were counted after each 3 min bioassay. The percentage of mosquitoes attracted to the baited port out of the total number of mosquitoes initially in the cage was recorded. Treatments consisted of: 1) the left hand of a male subject, 2) the left hand of a male subject with the addition of 50 µL of the Inhibitor on a porous inert slow release ceramic pad placed in the same port, 3)1 mL of a synthetic attractant blend (L-lactic acid, acetone, and dimethyldisulfide) on a slow release ceramic pad, and 4) 1 mL of the synthetic attractant plus 50 µL of the Inhibitor on a single slow-release ceramic pad.

Results: Figure 1 displays the dramatic reduction in attraction of mosquitoes due to release of the inhibitor in the same port as the attractive stimuli. The attractant blend by itself (92.7%) is highly efficient at collecting laboratory mosquitoes in the olfactometer. Releasing a small amount of inhibitor in the same port results in collection of only 12.8% of the mosquitoes in a 3 min period. Equally impressive is the reduction that occurs when the same amount of inhibitor is placed in the same port as a human hand. The hand alone averages 88.6% collection of the mosquitoes, while with the inhibitor present, the average collection is only 23.5%. The inhibitor has been tested with laboratory-reared *Anopheles albimanus* and *Anopheles quadrimaculatus* with results similar to these presented for *Ae. aegypti*. Although the mechanism of inhibition is not fully understood yet, the compound appears to influence at least two different behavioral aspects of mosquito host-seeking. It is evident that the presence of the inhibitor severely impedes the ability of mosquitoes to detect odors that would normally be highly attractive. Additionally, for those mosquitoes that are activated to flight, it impedes the ability of many of those mosquitoes to locate the source of the attractive odors.

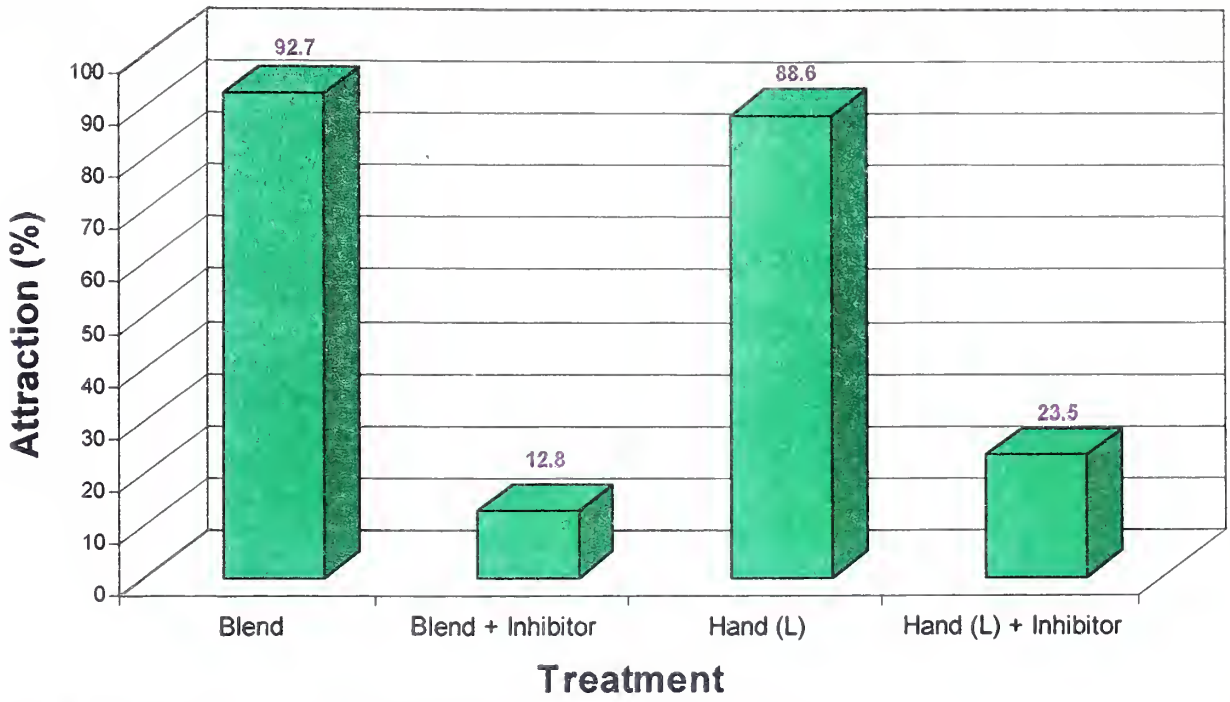


Figure 1. Attraction of *Aedes aegypti* to stimuli with and without an

CRIS 6615-32000-031-00D

EFFECT OF TYPE AND DEPTH OF HABITAT ON FORAGING BEHAVIOR OF FIVE SPECIES OF PARASITOIDS OF MUSCOID FLIES

C.J. Geden

Objective: Ecological niche partitioning by parasitoids that attack muscoid fly pupae in animal manure is still poorly understood. A better understanding of habitat preferences and utilization by these parasitoids could lead to improved matching of appropriate species to the target habitat in applied biological control programs. The depth at which parasitoids concentrate their search effort is an important ecological characteristic that may vary among parasitoid species. The objectives of the present study were to address the following questions: 1) How similar are the exotic species *Dirhinus himalayanus* and *Spalangia gemina* to the native species *Musicidifurax raptor*, *S. cameroni*, and *S. endius* with respect to locating pupae at different habitat depths? 2) Does the type of substrate (poultry manure, soil, fly rearing medium) affect parasitoid searching behavior? 3) Do parasitoids adjust their searching strategy when given a choice of depths at which hosts are present?

Methods: Three substrates were tested: poultry manure (41% moisture), "spent" fly larval rearing medium (39% moisture), and sandy soil collected from under calf bedding at a dairy farm (4% moisture). Five species of parasitoids were tested: *Dirhinus himalayanus* (from Morocco), *Spalangia gemina* (from Brazil), *Musicidifurax raptor*, *S. cameroni*, and *S. endius* (from Florida). Live house fly pupae were placed in the test substrates at 0, 1, 2, 4, & 6 cm from the top in 350 ml plastic cups. Two choice situations were used for bioassays. In Ò:no-choiceÓ assays, 5 female parasitoids were placed in a cup containing 50 pupae placed at a single depth and held for 24 hours (N=2 replicates of three cups per depth). In "choice" assays, 75 female parasitoids were released into a large box containing an array of cups with 50 pupae placed at different depths (one depth per cup as before). N=2 replicates of three cups per depth. Choice assays were conducted at the same time as the no-choice assays. Parasitoids were removed after 24 hr and the pupae held for fly and parasitoid emergence.

Results: None of the parasitoids were able to forage below the surface of sandy soil, suggesting that some pupae in regions with extensive coastal planes such as the southeastern U. S. are relatively protected from parasitoid attacks. In poultry manure, the *Spalangia* spp. and *D. himalayanus* found substantially more of the deeply buried pupae than did *M. raptor*. The fly larval rearing medium had friability and particle size characteristics that made it the easiest substrate for the parasitoids to navigate. *M. raptor* and *S. gemina* attacked proportionally more pupae near the surface than at greater depths in this substrate, whereas *S. cameroni* and *S. endius* tended to seek hosts at greater depths. The parasitoids generally did not alter their foraging algorithm regardless of whether they were given a choice of foraging opportunities. In summary, these indicate that *Muscidifurax* spp. concentrate their search efforts near the surface of the habitat whereas most *Spalangia* spp. tend to locate buried hosts, but these preferences are modulated by the physical properties of the substrate.

CRIS 6615-32000-031-00D

NOSEMA DISEASE OF THE ENCYRTID PARASITOID *TACHINAEPHAGOUS ZEALANDICUS*

C.J. Geden, M. Ferreira de Almeida, J.J. Becnel and C.K. Boohene

Objective: *Tachinaephagous zealandicus* is an encyrtid parasitoid of house flies and other muscoid Diptera inhabiting livestock and poultry manure, and is widely distributed in the southern hemisphere. The parasitoids attack mature fly larvae as they seek pupation sites, depositing multiple eggs in the hemocoel of the larva. The host pupates and continues developing slowly while the parasitoid larvae develop within the body cavity. The host is killed near the end of the parasitoidsÕ development, and the *T. zealandicus* immatures pupate within the mummified host remains. Development is completed in about 22 days at 25°C. A colony of *T. zealandicus* from Brazil recently was found to be infected with a microsporidian pathogen resembling *Nosema muscidifuracis*, a pathogen of the pteromalid parasitoid *Muscidifurax raptor*. The objectives of the present study were to evaluate transmission patterns, treatment, and impact of this pathogen on its parasitoid host.

Methods: Transmission tests were conducted to determine 1) whether the pathogen is transmitted from infected mothers to progeny; and 2) whether transmission occurs horizontally (among larvae) within hosts that have been parasitized by both infected and uninfected mothers. The effect of infection on development time was assessed by rearing infected and uninfected parasitoids at temperatures ranging from 15 to 30°C. Longevity of infected and uninfected parasitoids was determined at the above temperatures under two nutritional regimes, with the parasitoids given either water only or water plus honey. Attack rates and fecundity of healthy vs. infected parasitoids were examined using three host species: house fly, *Sarcophaga bullata* and *Chrysomyia putoria*. Heat shock and drug therapies (rifampicin and albendazole) were evaluated for managing the disease.

Results: Transmission testing indicates that the pathogen is transmitted transovarially. Horizontal transmission occurred when larvae of uninfected and infected parasitoids both occurred within superparasitized hosts, but the mechanism of this transmission is not known. Infected females had 30-50% shorter lifespans and produced about half as many live progeny as healthy females. Longevity differences were most pronounced in the parasitoids that were given honey (longevity was very short overall when parasitoids were only given water). Many of the progeny from infected mothers died in the immature stages within the dipteran hosts. Infection had no substantial effect on sex ratios or development time of the parasitoids. Heat shock was not effective for managing the disease because of the sensitivity of the host parasitoid to elevated temperatures. *Per os* administration of a 1.5% solution of rifampicin to adult *T. zealanidicus* resulted in reduced rates of maternal transmission. This reduction was sufficient to allow the isolation of clean females and the establishment of an uninfected colony of the parasitoid. The availability of healthy parasitoids will facilitate further evaluation of this parasitoid as a candidate for importation and release into the U.S. as a classical biological control agent.

CRIS 6615-32000-031-00D

FIELD EFFICACY TESTS OF SEVERAL ATTRACTANT BLENDS FOR MOSQUITOES AND BITING FLIES

D.L. Kline, U.R. Bernier and D.R. Barnard

Objective: A major emphasis of our current mosquito management research is the development of selective, environmentally friendly methods of control. One concept under investigation is the use of attractant-baited traps. This approach requires the discovery of new, effective attractants and blends of attractants, which have the potential to enhance collection size of mosquitoes and other biting flies compared to traps baited only with carbon dioxide (CO_2). The ultimate goal is the management of localized pest/vector populations without the need for intervention with chemical insecticides. The objective of this study was to evaluate 3 blends against natural populations which performed well in laboratory olfactometer tests against laboratory reared Aedes aegypti.

Methods: Field trapping experiments (a series of 4 x 4 Latin square experiments) were conducted at the Lower Suwannee, located near Cedar Key, FL, against natural populations of salt marsh and woodland mosquito species and associated biting fly species. The Counterflow Geometry (CFG) trap was used for all field trials. Traps were located ca. .1 mile apart. They were operated continuously for ca. 23 hr each day, beginning at 2 hr before sunset. Each day the traps were rotated to a new position. Each trap was baited with 500 cc/min CO_2 alone or 500 cc/min CO_2 in combination with one of 3 blends. The blend was released from a Quorpak^R jar (120 ml capacity, 40 mm mouth opening) suspended from the bottom of the CFG trap with the lid removed. CO_2 was supplied from 9 kg compressed gas cylinders supplied with a regulator. Three blends were used, designated in this study as Red, Blue and Green. The Red blend was composed of 400 ml acetone: 10 ml 1-hexen-3--ol: 10 ml 1-octen-3-ol; the Blue blend consisted of 400 ml acetone: 1 g/L lactic acid: 20 ml glycoxlic acid; the Green blend consisted of 400 ml acetone:20 ml dimethyl disulfide: 1.5 g/L lactic acid. Each day the blend mixtures were filled to the 120 ml level. A relative index of efficacy was determined by dividing each treatment trap collection counts by the trap collection count obtained from the trap baited with CO_2 alone.

Results: Trap collections consisted of several species of mosquitoes, Culicoides furens, Lutzomyia spp. (phlebotomine sandflies) and Diachlorus ferrugatus (tabanid). Traps baited with the Red blend resulted in collections greater than the CO_2 only baited traps for all groups of biting flies and mosquito species except for Culex nigripalpus. Culicoides furens collections were especially enhanced (52.5X) by the addition of this blend. Collections in traps baited with the Blue blend were reduced for all biting fly groups and mosquito species except for Aedes infirmatus which equaled the CO_2 only standard. Traps baited with the Green blend resulted in reduced collections for all mosquito species except Anopheles crucians; collection size of Culicoides furens (2.9X) and Diachlorus ferrugatus (1.5X) also increased slightly.

Table 1. Relative efficacy of traps baited with a combination of CO_2 + various blends of chemical attractants compared to CO_2 only baited traps for collections of mosquitoes and other biting flies. AeT = Aedes *taeniorhynchus*; AeI = *Aedes infirmatus*; AeTri = *Aedes triseriatus*; AnCr = *Anopheles crucians*; CxN = *Culex nigripalpus*; CuF = *Culicoides furens*; LUTZ = *Lutzomyia*; DF = *Diachlorus ferrugatus*.

Bait	All Mosquitoes	AeT	AeI	AeTri	AnCr	CxN	CuF	LUTZ	DF
CO_2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
+Red	2.5	3.1	1.8	3.4	2.9	0.2	52.5	7.9	2.9
+Blue	0.6	0.6	1.0	0.8	0.8	0.2	0.6	0.7	0.7
+Green	0.8	0.8	0.9	0.6	1.3	0.2	2.9	0.4	1.5

CRIS 6615-32000-032-00D

DIVALENT CATIONS ARE REQUIRED FOR TRANSMISSION OF A NEW BACULOVIRUS FROM THE MOSQUITO *CULEX NIGRIPALPUS*

J.J. Becnel, S. White, B. Moser, and T. Fukuda

Objective: To determine the requirements for transmission of a new microbial pathogen from *Culex* spp. mosquitoes (important encephalitis vectors) produced in agricultural wastewater.

Methods: *Culex nigripalpus* larvae infected with a new baculovirus (CuniNPV) were collected from a man made settling pond of swine effluent located in Gainesville Florida. Groups of 50 infected larvae were frozen in deionized water and held at -80°C. These infected larvae were used for laboratory bioassay. Standard bioassays were conducted with groups of 100 3-4 day old *C. quinquefasciatus* larvae (second instars) exposed in 3.5 oz plastic cups in 100 ml of water with 2 ml of 2% alfalfa and potbelly pig chow mixture (2:1). Infected larvae were homogenized in a glass tissue grinder and a concentration of 5-8 LE was used per exposure group. After 48 hrs, the larvae were removed and examined microscopically for signs of infection. Only those larvae with hypertrophied nuclei in midgut epithelial cells were scored as positive. Field water assays. Larvae were exposed to CuniNPV in whole field water and deionized water. The increase in percent infection was calculated based on paired tests with and without virus in deionized water and swine wastewater. Enhancement assays. Deletion analysis of the principle cations present in the field water was used to determine if salts were critical for transmission of CuniNPV. A salt mixture of 1.8 mM $MgCl_2$, 0.5 mM $CaCl_2$, 6.0 mM KCl, 1.8 mM NaCl and 3 mM NH_4Cl was used as an exposure media. Bioassays were conducted in the complete salt mixture, mixtures with one salt deleted and in each salt individually. Based on the results of these tests, additional assays with CuniNPV were conducted in 63% serial dilutions of 20 mM $MgCl_2$ to determine the effect of cation concentration on infection levels. To determine possible inhibition of transmission, assays were conducted in 10 mM $MgCl_2$ with 50% serial dilutions of 20 mM $CaCl_2$.

Results: There was an 80-fold increase in infection of *C. quinquefasciatus* larvae when exposed to CuniNPV in swine wastewater when compared to exposures made in deionized water. These results indicated that there were factors present in the swine wastewater that mediated transmission of the virus. The addition of a salt mixture similar to that present in the swine pond water to the CuniNPV assay significantly improved infections with an average infection rate in larvae of 11.0 %. The salts were also tested individually and in combination. The only individual salt that was essential for infectivity was magnesium with an average larval infection rate of 10.4%. Salt mixtures without Mg^{2+} resulted in less than 1.0 % infections. Elimination of K^+ and Na had little effect on the infection rate. With the exception of the complete salt mixture, the mixtures with Ca^{2+} tended to result in lower infection levels. Serial dilutions with Mg^{2+} showed that CuniNPV percent infection was positively correlated to the Mg^{2+} concentration: as Mg^{2+} concentrations increased, CuniNPV infections increased. Conversely, as Ca^{2+} concentrations increased in the presence of Mg^{2+}, infections decreased. Results of assays with other cations found that only divalent cations played a role as activators or inhibitors of infection.

CRIS 6615-32000-032-00D

COMPARISON OF VOLATILE ORGANIC ODORANTS IN HAY AND GRASS INFUSIONS WITH ODORANTS PRESENT IN FLUSHED DAIRY MANURE USING MODERN GC-MS

D.A. Carlson and A.C. Wilkie

Objective: Flushed Dairy Manure (FDM) is moved from barns to storage lagoons, creating a large volume of dilute manure to be used as fertilizer and irrigation water. Shorter FDM storage time saves money, but noxious odorants are released during this open-air digestion. Odorous manure and contaminated wastewater are associated with mosquito oviposition, fly nuisance and transmission of disease organisms by the housefly. Problems with nuisance odors has continued with swine and cow manure and with bacterial and offal sources. Generation of house flies generates lawsuits. House fly-vectored disease transmission of viruses has been confirmed as have bacteria reported to cause stomach ulcers in humans. *E. coli* strain O157:H57 was found in house flies and stable flies captured near cowsheds and associated by DNA testing with an outbreak of food poisoning at a nearby children's nursery. Massive numbers of bird-feeding mosquitoes are produced in lagoons containing decomposing manure. Recent African tests were reported with synthetic oviposition stimulant from *Culex quinquefaciatus* mosquitoes and skatole.

Methods: Purge and Trap (P&T) GC mass spectrometry (GC-MS) was used for large scale (30 ml) samples of FDM, hay and grass infusions as if sampling for EPA-type priority pollutants in water, employing a non-polar capillary column. Microscale purge and trap (Ms-P&T) was used for statically stripping the headspace of a 10 ml liquid sample with a small volume of helium onto a glass beads, then a porous polymer trap just before GC-MS. Solid-phase microextraction fibers (SPME) were used to sample headspace over FDM and infusion liquids for volatiles, and for sampling polar compounds in solution in which SPME-Liquid fibers were dipped then inserted directly into the GC injection port. This represents many of the volatiles present, but not all odorants such as biogenic amines, and a different technique (HPLC) must be employed for them.

Results: P&T Liquid showed 6 sulfur compounds in FDM while Ms P&T showed those and 8 more in FDM. P&T Liquid showed many of the same: methylmercaptan, dimethyl sulfide and dimethyl disulfide in hay, while Ms P&T showed carbonyl sulfide, methyl mercaptan, dimethyl sulfide, carbon disulfide, dimethyl disulfide and trace amounts of other sulfides in hay but less in grass (Fig. 1). SPME Headspace showed C2 to C7 organic acids in FDM, with similar results for grass and hay showing nearly 2x more of each C2 to C6 acid found in hay. SPME Liquid showed 3 to 7 times more acids in hay depending on the acid. The hay sample was also more odorous to the human observer for the following reasons. Phenol, m- and p-cresol were found at 11 mg/L with m- plus p-cresol at 38 mg/L by SPME Liquid in hay, about 1/4 as much in grass. SPME headspace showed about half as much at 1 mg/L in hay as grass. Curiously, indole and skatole were found in FDM at about 1 mg/L but not in grass or hay.

The problem is to puzzle out of the lists a mixture of active component(s) that flies and mosquitoes respond to with movement, feeding, aggregation and oviposition. It is well known by electroantennagram studies that the antennae of flies and mosquitoes detect many of these compounds, but detection does not necessarily mean that a particular behavior will result.

Figure 1. Grass infusion analyzed by GC-MS using Purge and Trap (Liquid).

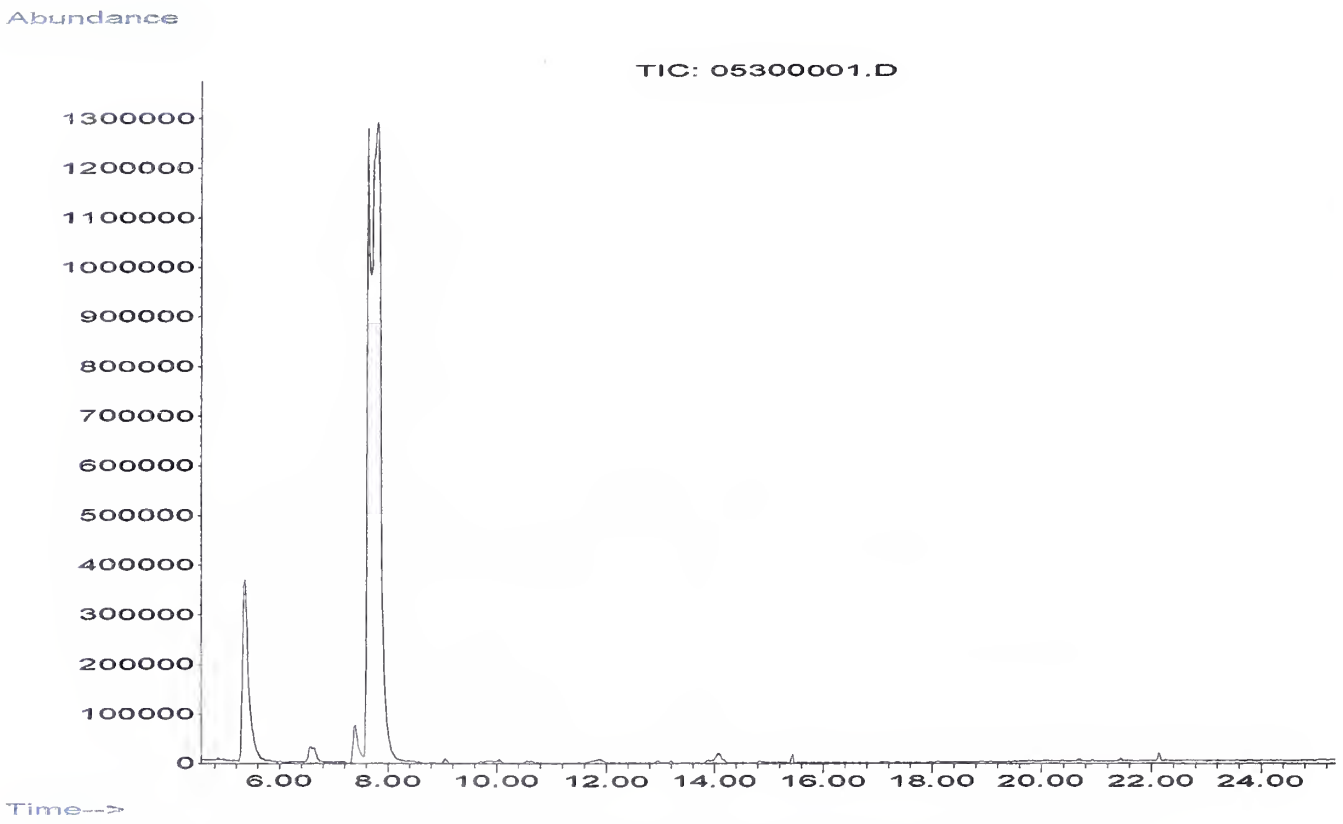
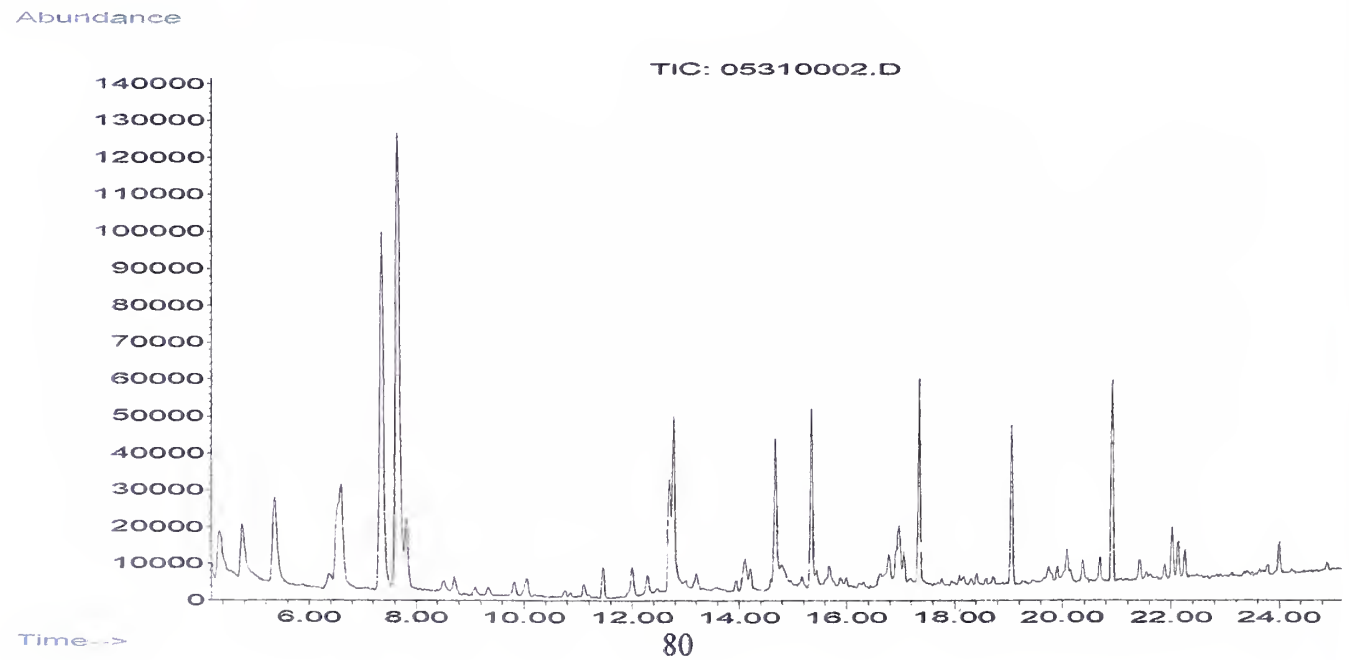


Figure 2. Hay infusion analyzed by GC-MS using Purge and Trap (Liquid)



CRIS 6615-32000-032-00D

EVALUATING SELECTED DILUTIONS OF COMMERCIALLY AVAILABLE HOUSE FLY ATTRACTANTS

J.A. Hogsette and D.A. Carlson

Objective: Commercially available house fly attractants are widely used in agricultural situations to lure house flies to traps. In these situations, where animal and feed odors are pronounced, a malodorous attractant goes unnoticed. By comparison, strong smelling attractants would be unsuitable for use in indoor areas frequented by humans. Therefore, tests have been done to determine how much malodorous attractants can be diluted and still be attractive to flies under laboratory conditions.

Methods: A laboratory (~12 x 16 ft) fitted with cupboards and counters was used for testing. Attractants were applied to small strips of filter paper which were then affixed to sticky 3 x 5 cards. Cards were placed on prepared stations about 30 cm above the floor, with one card on each side of the room. Approximately 50 mixed-sex 5- to 7-day-old house flies were released into the room when a test began. Flies caught on cards were counted at three intervals during a 22-hr test period. Cards with attractants were replaced after each 22-hr test period and a new group of flies was released.

Results: Testing is still underway, but at this point preliminary results indicate that the products are still highly attractive to house flies when diluted by several powers of 10. Even at full strength, the attractants are used in such small quantities that we are unable to detect their presence in the testing laboratory. There is a positional effect in the room which must be due either to airflow or lighting, although there is no discernible difference in these factors. Most flies were usually caught between the second count (ca. 9 hr after release) and the last count (ca. 22 hr after release), but reasons for this are unknown and speculation at present would be premature. Most flies captured on the sticky cards were females.

MORPHOLOGICAL AND MOLECULAR EVIDENCE FOR A NEW MOSQUITO BACULOVIRUS FROM *CULEX NIGRIPALPUS*

B.A. MOSER, J.J. Becnel and S. White

Objective: To provide the first morphological and molecular characterization of CuniNPV, a new mosquito baculovirus isolated from the encephalitis vector *Culex nigripalpus*.

Methods: *Culex nigripalpus* larvae infected with CuniNPV were collected from a man made settling pond of swine effluent located in Gainesville Florida. The virus was amplified in larval mosquitoes, harvested and purified on a Ludox gradient. Groups of 100, 3-4 day old *C. quinquefasciatus* or *C. nigripalpus* larvae were exposed to 20 LE virus in 3.5 oz plastic cups in 100 ml of water with 10 mM $MgCl_2$ plus 0.0 2% alfalfa and potbelly pig chow mixture (2:1). For phase and electron microscopy, midguts were dissected from exposed and unexposed larvae in Ringer's solution at various times postexposure. Cytopathological effects were determined by examination of mounted midguts with phase-contrast microscopy. Midguts were prepared for ultrastructural examination by standard protocols. Genomic libraries were generated by shotgun cloning of viral DNA, restricted with several enzymes, into pUC19. Recombinant plasmids were purified with Qiagen plasmid miniprep kits. DNA templates were sequenced from both ends with universal forward and reverse primers using dideoxy chain terminator sequencing.

Results: The morphological and molecular data from this study indicate that CuniNPV belongs in the Baculoviridae but is distinct from the genera *Nucleopolyhedrovirus* (NPVs) and *Granuloviruses* (GVs). It has a double-stranded DNA genome of approximately 105-110 kbp (presumed to be circular) which is packaged into a rod-shaped enveloped capsid . The nucleocapsids average 40 nm in diameter and 200 nm in length. It has two virion phenotypes, an occluded form (ODV) that initiates infection in the midgut epithelial cells, and a budded form (BV) that spreads the infection in the midgut. Each ODV contains one nucleocapsid (singly-enveloped nucleocapsid). DNA replication occurs in the nucleus. Several genes diagnostic of members of the family Baculoviridae have been identified. These include *lef1*, *lef4*, *odv-e56* and *p74*. Like NPVs, the occlusion bodies (OBs) are found exclusively in the nuclei of infected cells during all stages of infection and replication. Unlike NPVs, they are globular, not polyhedral, in shape. They are similar in size (average diameter of 400 nm) to GV OBs. Each OB typically contains 4 virions as opposed to GVs where each OB contains one or two, rarely more virions. *C. nigripalpus* baculovirus gene order is distinct, and phylogenetic analysis of the putative p74 and DNA polymerase polypeptides placed the *C. nigripalpus* baculovirus into a separate taxon basal to the NPVs and GVs. These data show that CuniNPV is a baculovirus with unusual characteristics. Pending additional data, we have elected to retain it in the genus *Nucleopolyhedrovirus* but suggest that it may represent a new genus within the family Baculoviridae.

POSTHARVEST

AND

BIOREGULATION

CRIS - 6615-43000-007-00D--Population Management of Insects to Protect Stored Products

CRIS - 6615-43000-008-00D--Detection and Population Estimation of Stored Product Insects

CRIS 6615-43000-007-00D

INSECT INFESTATION OF A BOTANICALS WAREHOUSE IN NORTH-CENTRAL FLORIDA

R.T. Arbogast, P.E. Kendra, R.W. Mankin, and R.C. McDonald

Objective: Botanicals are crude vegetable medicinals consisting of roots, leaves, bark, or other plant materials that are sometimes used directly, but are generally refined and processed to produce pharmaceuticals and herbal supplements (nutraceuticals). Familiar examples include ginseng, gingko, St. John's wort, and saw palmetto. Use of herbal supplements has grown markedly in recent years, and the economic value of botanicals used annually in their production is estimated at $300-500 million. Harvested plant materials are dried and stored before processing. During storage they are subject to damage and contamination by stored-product insects. We studied insect infestation in a warehouse used alternately for storage of saw palmetto berries and passion flower. Our objective was to gain a better understanding of insect problems associated with stored botanicals and to identify measure that could be taken to mitigate the problems.

Methods: We monitored insect populations in the warehouse intermittently from April 1998 - May 1999. Moths were monitored with pheromone-baited sticky traps (SP-Locator traps with Minimoth lures, AgriSense, Mid Glamorgan, UK), and beetles with pitfall traps (FLĪT-TRAK M^2, TRÉCÉ, Salinas, California) baited with *L. serricorne* and *Tribolium* pheromones and a food attractant oil (furnished with the traps). An array of traps was deployed in the warehouse with a moth trap and beetle trap at each location. Number of traps and frequency of observations varied. Temporal variation of insect populations was examined using numbers captured per unit trapping effort, which was expressed as trap days (number of traps X number of days deployed).

Results: Eighteen species of stored-product pests, as well as predators, parasites and accidental invaders, were found in the warehouse. The almond moth, *Cadra cautella* (Walker), and the cigarette beetle, *Lasioderma serricorne* (F.), were the prevalent pest species. The merchant grain beetle, *Oryzaephilus mercator* (Fauvel), the hairy fungus beetle, *Typhaea stercorea* (L.), the Indianmeal moth, *Plodia interpunctella* (Hübner), and the red flour beetle, *Tribolium castaneum* (Herbst) were also abundant. Temporal variation in rates of capture suggested differences among species in their ability to infest saw palmetto and passion flower. This variation is illustrated for the two major pest species in Fig. 1. It suggests that the almond moth develops well on saw palmetto, but poorly on passion flower. Rate of capture was highest during the spring, when saw palmetto had been in storage for several months. The cigarette beetle appears to develop well on both commodities. Rate of capture increased through the summer and fall on passion flower and then on saw palmetto. There were no captures during February and March 1999, but captures resumed in April and May. Although winters at the site are mild, temperatures may have been low enough to reduce activity and slow development. Free air temperatures recorded at a weather station 10 miles away showed monthly mean minima below 15°C for December-March, but the lowest mean monthly maximum was 23.5°C in January. Temperatures in the warehouse would have been above ambient, especially on sunny days.

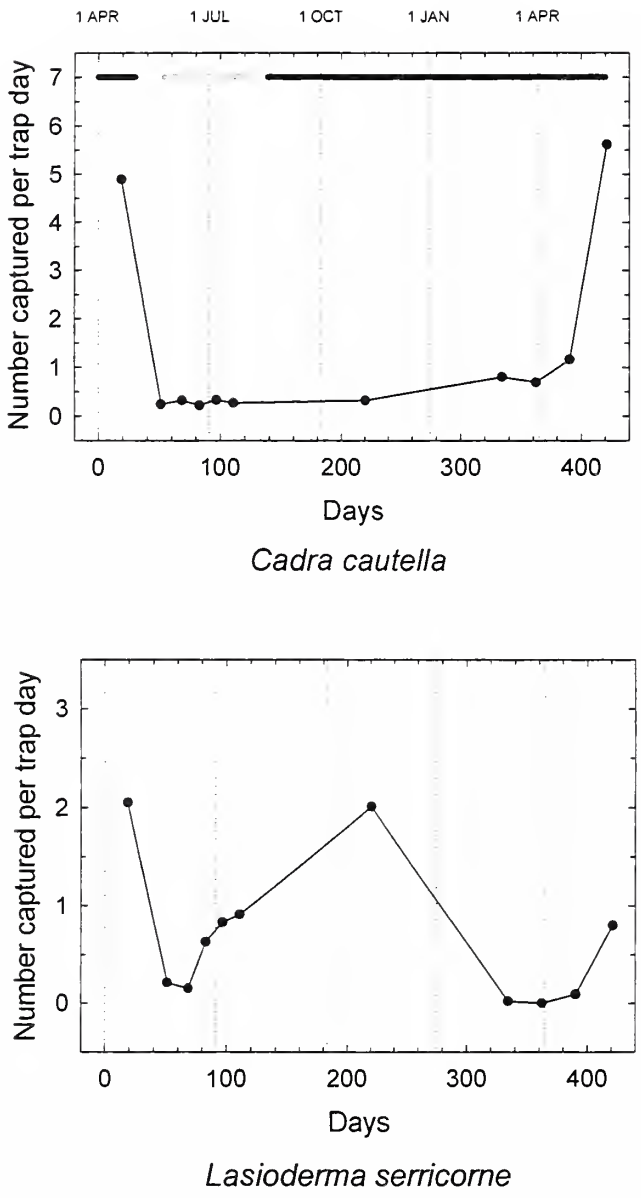


Figure 1. Temporal variation in rates of trap capture of the two major pests in the warehouse. The horizontal line across the top of the upper graph indicates the contents of the warehouse. Black is saw palmetto and gray is passion flower. Gaps in the line show when the warehouse was empty.

CRIS 6615-43000-007-00D

A DENSOVIRUS-DERIVED VECTOR FOR THE RAPID ASSESSMENT OF PROMOTER ACTIVITY IN INSECTS.

H. Bossin and P.D. Shirk

Objective: Identification of transposon mediated transformation events in many non-dipteran insects depends in part on the availability of promoters that drive high, specific expression of marker genes. In order to develop a convenient somatic transformation system to test promoter activity in insects, we utilized the ability of the *Junonia coenia* lepidopteran densovirus to integrate into the genome of the host insect. By incorporating an expression cassette into the vector pJDsRedH we can test the functionality of a promoter and establish its phenotype before utilizing it as a transformation marker.

Methods: pJDsRedH is a defective densovirus genome with an insertion of the DsRed DNA into the viral structural proteins coding sequence. Various expression cassettes including the 3xP3EGFP (Wimmer et al., Nature 402, 370-371) and PubEGFP (Handler & Harrel, Insect Mole. Biol. 8, 449-457) have been cloned into the pJDsRedH, and the resulting vectors were injected into fruitfly, *Drosophila melanogaster*, and Indianmeal moth, *Plodia interpunctella*, eggs. While the maintenance of the viral genome was monitored by the expression of the DsRed fluorescent protein, the G0 larvae and adults were scored for green fluorescent protein expression in the expected tissues.

Results: We have developed a system based on the somatic transformation properties of the *Junonia coenia* lepidopteran densovirus and designed the vector pJDsRedH. This vector allows for convenient phenotype evaluation of the intended marker expression cassette in the insect species of interest and alleviates the need for achieving concurrent development of a transgenesis vector and a genetic marker system for an insect. Following microinjection of the pJDsRedH vector into embryos, the larvae were screened for the expression of the DSRed fluorescent protein. Those larvae showing DSRedFP were maintained and examined for co-expression of green fluorescent protein in the targeted tissues through out the larval, pupal and adult stages. Results for the pJDsRedH/3xP3EGFP plasmid showed co-expression of the DSRedFP and EGFP in the ocelli and eyes for both the fruitfly and Indianmeal moth larvae and adults. The results demonstrate that the pJDsRedH vector can be used successfully to test functionality of even highly tissue specific promoters in Drosophilid and non-drosophilid insects.

CRIS 6615-43000-007-00D

DEVELOPMENT OF THE PIGGYBAC TRANSPOSON AS A GENE VECTOR FOR INSECTS

H. Bossin and P.D. Shirk

Objective: The ability to genetically modify insects depends upon biotechnology to efficiently introduce a foreign gene construct into the genome of a host insect. The *piggyBac* transposon, which is a mobile genetic element, has been modified as a gene vector system to genetically transform agricultural pest Lepidoptera, Coleoptera and Diptera. We established protocols to optimize procedures and conditions for the use of the *piggyBac* transposon as a gene vector system in Lepidoptera and Diptera. The factors assessed were the timing and conditions of embryo injections, the type of helper plasmid, the concentration of vector and helper plasmid and the activity of promoters and marker genes.

Methods: The insects tested were white eye mutant strains of the Indianmeal moth, *Plodia interpunctella*, the cabbage looper, *Trichoplusia ni*, and the fruitfly, *Drosophila melanogaster*. The *piggyBac* gene vectors tested were pB3xP3EGFP (Wimmer et al., Nature 402, 370-371) which results in ocelli and eye specific expression of green fluorescent protein (GFP), and pPIGA3GFP (Tamura et al. Nature Biotech. 18: 81-84) which results in expression of GFP in all tissues. The helper plasmids tested were phsp-pBac (Handler & Harrel, Insect Mole. Biol. 8, 449-457) which has a heat shock promoter to increase the expression of the *piggyBac* transposase and pOPhsp70wc which has both inverted repeat termini removed. Larvae, pupae and adults were screened for transformation by the expression of GFP in G1 progeny.

Results: A novel procedure for the microinjection of *Drosophila* eggs that permitted the microinjection of large numbers of eggs that were less than 20 min old without dechorionation or desication was used. Using the *D. melanogaster wm* strain with the pB3xP3EGFP vector and phsp-pBac helper and varying the concentration of the vector and helper and the timing of the embryo injections, we have achieved transformation rates where over 60% of the G0 reproductive adults produced genetically transformed G1 progeny. In several of these line up to 100% of G1 progeny were transformed. Southern analysis of the genomic DNA from these strains showed the presence of multiple *piggyBac* insertions in the strains examined. This provides a highly efficient method for the genetic transformation of *Drosophila*. When testing the pB3xP3EGFP vector in combination with the pOPhsp70wc helper, the removal of the 3' terminal repeat in the pOPhsp70wc helper appears to have blocked the production of active transposase because no transformed progeny were recovered using this construct. Similar tests were conducted in the Indianmeal moth and the cabbage looper without the detection of a successful transformation event.

CRIS 6615-43000-007-00D

JUVENILE HORMONE PROMOTES CHANGES IN CELL SHAPE SIMILAR TO THOSE ELICITED BY COMPOUNDS THAT ACTIVATE PHOSPHOLIPASES C AND D1, AND RHO GTPASES

S.D. Dyby, C.E. Leach, and H. Oberlander

Objective: To determine the impact of juvenile hormone (JH) on cell shape, survival, and proliferation in a lepidopteran cell line, and to compare its impact with compounds known to activate specific signal transduction pathways.

Methods: An Indian meal moth cell line, established in this laboratory from wing imaginal discs, was used to determine the response of cells to JH, bioactive lipids, enzymes, and growth factors. The cells were maintained in an antibiotic-free Grace's medium supplemented with 10% fetal bovine serum. Experimental subcultures were maintained for 20 hours in serum-free medium before exposure to JH or other compounds. After incubation with the test compounds, the cells were returned to Grace's medium supplemented with 10% fetal bovine serum and kept for observation.

Results: JH I and III (and methoprene and fenoxycarb) promote the maintenance of lamellipodia in a lepidopteran cell line, and mimic the impact on cell shape caused by adding lysophoshatidic acid (LPA), exogenous phospholipase D, linoleic acid, or basic fibroblast growth factor-4, but not cis-9-retinoic acid, bone morphogenesis protein-4, ceramide C6, or oleic acid. Even 04 ng/ml JH I is sufficient to cause a doubling of cells with lamellipodia (seen as thin, flat cytoplasmic extensions) over that of controls, with the effects remaining up to several days after test compounds are removed. JHI (0.4 ng/ml - 16 µg/ml) and JHIII (0.1 - 40 µg/ml), phospholipase D (0.5 - 3 units/ml), and linoleic acid (10 -200 µg/ml) induce significantly greater numbers of cells to form lamellipodia. In contrast, the cellular response to bone morphogenesis protein-4 (0.1-4 ng/ml), ceramide C6 (10 µM), 9-cis-retinoic acid (1 µM) or oleic acid (100-200 µg/ml) was not significantly different from controls, and the percent of cells with lamellipodia was consistently lower than controls. LPA, exogenous phospholipase D, and basic fibroblast growth factor all activate intracellular phospholipases C and D1, and Rho GTPases (Cross et al., 2000; Exton, 1999) that lead to reorganizing of the cytoskeleton, altering of the cell cycle, and upregulating early response genes. Ceramide C6 downregulates intracellular phospholipase D1 activation, and both cis-9-retinoic acid and bone morphogenesis protein-4 signal via cyclic AMP, unlike LPA and exogenous phospholipase D. Oleic acid does not activate Rho GTPases. The results for JH were therefore consistent with compounds that activate phospholipases and Rho GTPases, and not with those that signal via other pathways.

CRIS 6615-43000-007-00D

DETECTION AND POPULATION ESTIMATION OF STORED PRODUCT INSECTS

N.D. Epsky and D. Shuman

Objective: Meetings were held with members of the Electronic Grain Probe Insect Counter (EGPIC, U.S. Patent No. 5,646,407) Working Group to discuss modifications to the prototype EGPIC probe traps that should be incorporated into new commercial EGPIC probe traps. All parts of the electronic probe traps will be manufactured by a commercial producer, including the upper probe body. The prototype EGPIC probe's upper body is a commercially available grain probe trap, which is a cylinder with a 14-cm-long trapping surface that has 180 evenly spaced holes. Working group members recommended that the trapping surface be increased to 40-cm-long. Research was conducted to determine the relationship between hole number and hole spacing, and target insect capture; and to compare capture in the prototype EGPIC probes with capture in EGPIC probes that have manufactured upper bodies.

Methods: Test upper probe bodies were produced from PVC cylinders that were the same diameter and hole size as the commercially available grain probe traps, but with a 40-cm-long trapping surface. One set of upper probe bodies had six columns and 10, 21 and 41 holes per column for a total of 60, 126 and 246 holes, respectively. A second set of upper probe bodies had 12 columns and 5, 11, 21 and 41 holes per column for a total of 60, 132, 252 and 492 holes, respectively. These were tested against the prototype upper body that 12 columns and 15 holes per column, for a total of 180 holes. Tests of probe bodies were conducted in laboratory trials using 2-3 week old adults of the sawtoothed grain beetle, the rice weevil and the red flour beetle. Tests were conducted with 2.8 kg wheat and 200 insects in mini-silo cylinders (10 cm diameter by 50 cm tall) that contained a single probe placed in the center. Number of insects captured was recorded electronically for 72 h.

Results: There was a direct relationship between number of holes and percent of insects captured in tests of sawtoothed grain beetles and rice weevils, but number of holes had no effect on percent of red flour beetles capture. However, probes with 12 columns of holes captured more red flour beetles than probes with the same number of holes but only six columns. There was no difference in percent capture among the 12-column probe bodies with either 252 or 492 holes, or the prototype EGPIC probe with 180 holes. For the final commercial upper probe body design, the number of columns was reduced to 10 to accommodate the electronic cables from the infrared diodes. For the initial production run of the commercial EGPIC probes, the upper probe body will be a 40-cm-long trapping surface with 10 columns and 21 holes per column, for a total of 210 holes. Thus, the trapping surface area in the commercial upper probe body has been increased to provide greater coverage in the grain and the total number of holes slightly increased. Laboratory tests are underway to compare the new 210 hole commercial EGPIC probe design with the prototype 180 hole EGPIC probe.

CRIS 6615-43000-007-00D

DEVELOPMENT OF AN ACOUSTIC SYSTEM FOR DETECTION OF BLACK VINE WEEVIL IN COMMERCIAL ORNAMENTAL NURSERIES

R.W. Mankin

Objective: The black vine weevil (BVW), *Otiorhynchus sulcatus*, is an important pest affecting the nursery industry in the Northwest. The costs of scouting for insects and discarding infested plants could be greatly reduced if the insects were detected soon after potting. A portable acoustic system originally developed at CMAVE to detect hidden infestations of insects in grain has been adapted to detect insects moving and feeding in soil. The system was tested in two studies with Dr. James Fisher at the USDA-ARS Horticultural Crops Research Laboratory in Corvallis, OR as a potential monitoring tool for the nursery industry to use in detecting infestations early in the growing season. Our objective was to determine if recently hatched, small larvae could be detected acoustically and distinguished from background noise in the fall of the year when scouting normally occurs.

Methods: The acoustic system contains a vibration sensor that detects insect movement and feeding activity in the root system, an amplifier, and a recorder with headphones. The insect sounds can be identified by an experienced listener at the time of monitoring, and the recorded signals can be analyzed in the laboratory using custom-written signal processing software. In a laboratory study, 21 1-gal pots containing popular nursery plants, including Rhododendron, strawberry, Irish yew, and Alberta Spruce, were exposed to natural infestations of BVW. A field study at the Monrovia, Inc., nursery in Dayton, OR, included 17 naturally infested pots containing blue spruce or Boston ivy. The pots were monitored with the acoustic system, the roots were examined for confirmation of the presence or absence of BVW, and any insects found were classified and weighed. The recorded signals were subsequently analyzed in the laboratory.

Results: We determined that BVW larvae were large enough to be detected by the acoustic system during late fall in both the laboratory and the field. Eighteen of 38 pots produced negligible sound and were confirmed to contain no insects. The other 20 pots contained one or more BVW larvae weighing 3-80 mg. All but 2 of these pots produced sounds that both the listener and the computer program identified as insect sounds. In the 2 false-negative cases, neither the listener nor the computer detected any sounds, and it is likely that the insect did not move during the recording period. A library of BVW sounds was constructed for use in training scouts at commercial nurseries who could use the system as an early warning device. Efforts are in progress to develop a less complicated device for use by nontechnical personnel.

DETECTION AND POPULATION ESTIMATION OF STORED PRODUCT INSECTS

D. Shuman and N.D. Epsky

Objective: To develop and evaluate automated systems for monitoring hidden insect infestations in stored commodities.

Methods: a) Development has continued on the refinement of the Electronic Grain Probe Insect Counter (EGPIC - U.S. Patent No.5,646,404 issued 7/97) that electronically counts the number of insects that fall through it as a means of monitoring infestations in stored-products. The detrimental effect of internal infrared-light reflections (diffusing the beam) in the sensor head prompted a study of the infrared reflectivity of various materials and surface treatments. To accomplish this, a surface reflectance tester was developed and constructed. The sensor head design was modified to permit machining from a single piece of plastic. The spatial characteristic of the infrared beam intensity was mapped and insect drop tests were conducted to produce sensor peak response histograms. In an effort to develop a method of calibrating electronic probes, precision carbide balls (0.3 - 1.0 mm) were dropped through the center of the infrared beam.

b) Development has continued on the Serial Multiplexing Addressable Register Transmission System (SMARTS - U.S. Patent No.5,907,559 issued 5/99) that can efficiently transmit data from over a million monitoring sensors (e.g., EGPIC, acoustic, temperature, etc.) distributed throughout a storage facility back to a central computer. A ten node tree structure including a 1200 m link was constructed for a field test to measure SMARTS transmission error rate. The SMARTS software was developed with a user friendly interface and menu options to customize itself for any hardware topology. A race condition in the Smarts Universal Node (SUN) circuit was discovered and repaired with a hardware modification.

Results: a) Tests with 13 different plastic materials, including the PVC and Delrin used in EGPIC prototype sensor heads, indicated that extruded black nylon 6/6 had the least infrared reflectivity. Further tests with six different surface treatments on nylon samples indicated that sand blasting provided the greatest reduction in infrared reflectivity. Therefore, the single piece sensor head will be sand blasted on its inner surface except for the bottom of the top funnel which will be masked during the sand blasting operation. The spatial variability of the infrared beam intensity accounted for the large breadth as of the sensor peak response histograms. Tests with the precision balls had narrow response histograms and also showed excellent response linearity as a function of cross sectional area. The 0.4 mm ball will be used to calibrate probes to have an 80 mV sensitivity threshold level which will ensure a 98% counting accuracy with rusty grain beetles.

b) The ongoing SMARTS field test has demonstrated high transmission accuracy with a 9600 baud rate. Occasional large induced voltages on the 1200 m link routed around agricultural fields caused damage to the connected SUN circuit boards indicating the need for improved methods of shielding the cables and protecting the circuit board components.

CRIS 6615-43000-007-00D

TECHNOLOGY TRANSFER OF THE ELECTRONIC GRAIN PROBE INSECT COUNTER

D. Shuman and N.D. Epsky

Objective: To aid in the technology transfer of the Electronic Grain Probe Insect Counter (EGPIC) System.

Methods: The Electronic Grain Probe Insect Counter (EGPIC - U.S. Patent No.5,646,404 issued 7/97) is a system developed to monitor infestations in stored-products. It uses infrared-beam sensors to electronically count the numbers of insects that crawl into and fall through perforated tubes distributed throughout stored agricultural products, and then displays and records these counts at a central computer. In order to help transfer this technology and aid in its commercialization, several outreach efforts were made. The EGPIC Working Group was established to expand EGPICs use in research, to field validate its potential as a stored-product pest management tool with a variety of commodities over a range of geographic locations, and to increase its exposure to the agricultural industry. The president of OPI Systems of Calgary, Canada, a company that develops and distributes technology for stored grain management, was invited to the ARS Workshop on Management of Stored Product Insects held in Manhattan, KS, October 1999. The EGPIC technology was showcased at the Technology 2009 Exposition in Miami Beach, FL, November, 1999.

Results: These outreach efforts led to OPI Systems' interest in commercializing the EGPIC technology. A CRADA was established with OPI Systems to refine EGPIC, make it compatible with their existing grain management technology, and put it into a form suitable for commercial manufacture. As a part of this CRADA, a trip was made to OPI Systems to confer with their engineering staff on the commercial EGPIC design. OPI Systems is currently negotiating with ARS for an exclusive license of the EGPIC technology. The CRADA is also supporting the development of new insect monitoring technologies.

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PREVENTING FLOUR MOTH INFESTATIONS OF STORED COMMODITIES: ALTERNATIVES TO HARD PESTICIDES

D. Silhacek and C. Murphy

Objective: To develop alternative procedures for managing insect pests that infest stored commodities so as to minimize or eliminate the hazards associated with conventional pesticide applications.

Methods: As a starting point, we broadly defined the fundamental requirements for a successful infestation of a food product stored in a clean warehouse: 1) Attraction of the female insect to the commodity; 2) Oviposition of viable eggs on the commodity; 3) Growth and development of the hatchling larvae on the commodity. Using the Indianmeal moth infestation of packaged commodities during storage in a clean warehouse as a model, we examined each of these parameters for vulnerabilities that would effectively prevent the infestation of the commodity.

Results: Our studies are summarized for each of the three parameters:

Attraction to Commodity: Warehouse tests indicate that female flour moths are attracted to a range of commodities (Figure 1, 2), but there are clearly species-specific preferences. Some commodities can contain species-specific repellents as well as attractants. The attraction of moths to a commodity does not ensure that eggs will be deposited directly on the commodity. In fact, the majority of eggs are strewn around the commodity but, in a clean warehouse, only a few of the newly hatched larvae have the energy reserves to travel more than a few centimeters to the commodity. Hence, the infestation arises from those eggs laid on the commodity and the commodity container. A dirty warehouse promotes crawl-in infestations from longer distances because of the nutrients in the debris.

Oviposition and Embryonic Development: Laboratory tests indicate that low levels of juvenoid agonists interfere with embryonic development when administered early in flour moth embryogenesis. We found that the agonist could be delivered to the developing embryo by contact of the gravid female or her eggs with juvenoid-treated surfaces. Tests indicate that a single juvenoid agonist application on the warehouse walls and the outer commodity packaging (box/case) can prevent commodity infestation for as long as a year (Figure 2; Figure 2, Inset).

Nutritional Quality of Commodity: In a warehouse, the success of a commodity infestation ultimately relies upon the nutritive credentials of the commodity. Processed cereal products currently in the market place range from being unsupportive to fully supportive of insect growth. Some of the commodity determinants that affect insect development are water content, nutrient availability, palatability, texture and hardness. One or any combination of these determinants could be incorporated into a processed commodity to restrict insect infestations. It is our aim to develop a formula for processing cereal products that makes them unsuitable for insect infestation during warehouse and retail storage.

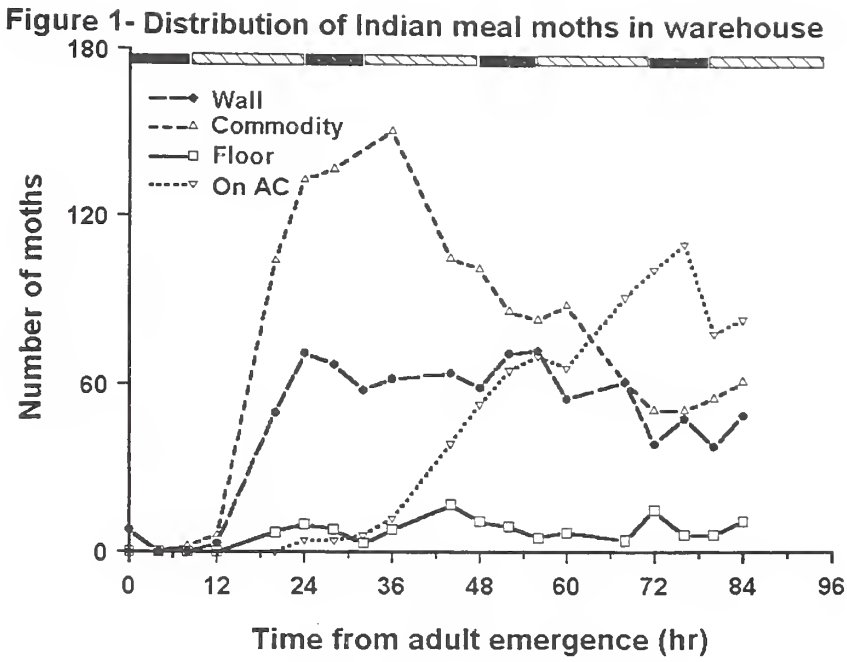

Figure 1. Distribution of Indianmeal moths over time when 5- to 6-day old pupae are introduced into a clean warehouse at 0 hours. AC is the foam inlet to the window air conditioner; the outlet was located just above inlet.

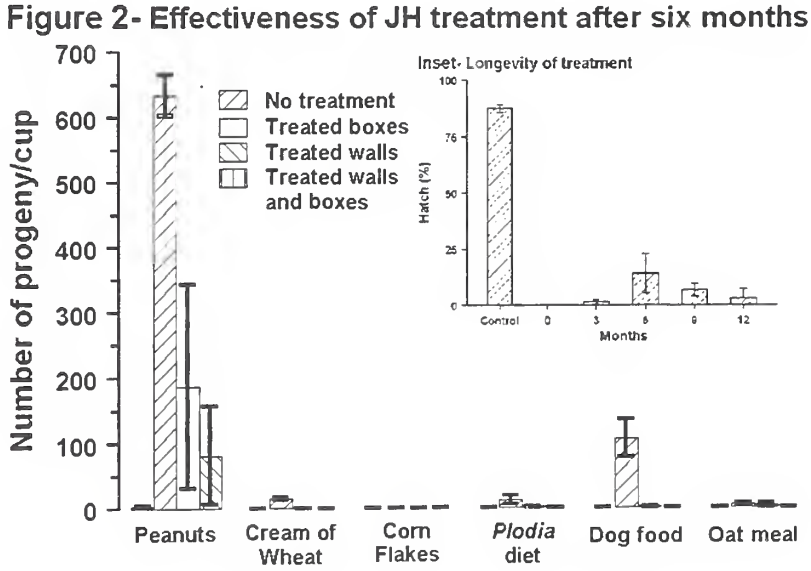

Figure 2. Infestation of commodities stored in a warehouse when Fenoxycarb is applied warehouse walls, commodity boxes or both six months prior to assay. Inset shows that treatment on warehouse wall panels is effective one year.

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